ICE 2012 DAEGU KOREA

XXIV International Congress of Entomology

'New Era in Entomology'

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ABSTRACTS

Abstracts compiled from the 24th ICE Congress website by Ohseok Kwon, Kyungpook National University. They are listed by number, in the order they appear in the Program book: Plenary talks PL1-PL6; Symposia S101M01-S1706TH06; General talks 0101M01-0703F16, and General posters PS1M001-PS7TH0478. Page numbers have been inserted (James Ridsdill-Smith, CSIRO Australia 8 March 2020).

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PL1

From individuals to populations: A tale of swarms, cannibals, ageing and human obesity

Stephen Simpson
University of Sydney, Australia

There can be few more important challenges in modern biology than explaining how the phenotypic features of organisms contribute to the populations, communities and ecosystems within which they exist, and how these in turn respond to changing environmental conditions.
The phenotype sits at the confluence of influences arising from the genes and the environment and it is the phenotype which is subject to natural selection. I will use my group's work on locusts and other insects to show how spanning individuals to ecosystems can be achieved by combining theory, laboratory and field experiments and by using techniques from a range of disciplines, including molecular biology, neurophysiology, biochemistry, behaviour, mathematics, statistical physics, computer science, engineering, evolutionary theory and landscape ecology.
Locust plagues are one of the most infamous insect scourges, affecting the lives of 1 in 10 people on the planet. But they have also provided important new clues into the causes of human obesity, how we age, and the complex behaviour of crowds.
I will take us on a strange journey that begins in the midst of a locust swarm and ends with the human obesity epidemic. Along the way you will see what you can discover by ticking a locust's leg with a paintbrush, how recreational drugs turn shy solitary locusts into swarming party animals, how robotic aircraft are being used to track swarms, how statistical physics offers the key to understanding collective movement, the sinister role played by cannibalism in marching bands of locusts and Mormon crickets, and how a powerful appetite for protein can help explain not only locust mass marching but also human obesity and ageing.

Keywords:*Nutrition*

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PL2

Biodiversity, Symbiosis and Evolution

Takema Fukatsu
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In nature, organisms are living in close association with surrounding physical environment as well as other organisms. Thus, each of the organisms is regarded as a component of the ecosystem. Considering the diverse microbial community found inside the organisms in general, each of the organisms can also be regarded as constituting an ecosystem.
Many insects and other organisms constantly harbor microorganisms inside their body, which has been referred to as "endosymbiosis". Due to the close spatial proximity, extremely intimate biological interactions and inter-dependency are commonly found between the partners called host and symbiont. Novel biological properties are often generated through such associations. In many cases, host and symbiont are integrated into an almost inseparable entity.
Our main research targets are diverse endosymbiotic interactions found in insects. We are also interested in sophisticated biological interactions associated with such phenomena as parasitism, reproductive manipulation, morphological manipulation, animal sociality, etc. Mechanisms underlying these biological interactions are investigated by using multi-disciplinary approaches including molecular biology, genetics, physiology, ecology and evolutionary biology.
Here I present an overview of our research topics on the endosymbiosis and evolution in insects, which underpin the overwhelming biodiversity of the animal group.

Keywords:*Insects, symbiosis, bacteria, mutualism, parasitism, evolution*

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PL3

Pathogen transmission dynamics at the human-mosquito interface

Thomas Scott
University of California, U.S.A.

Mosquitoes transmit pathogens that cause malaria, filariasis, dengue, yellow fever, West Nile fever, Rift valley fever, chikungunya, and many other infectious diseases that take an enormous toll on the health of humans, domestic animals, and wildlife. Efforts to prevent these diseases have been sorely tested by their resilient responses to our control efforts and the complexity of their transmission systems. After more than a century of trying to control them it is fair to ask, what are the key points where transmission is most vulnerable to operationally and cost effective intervention? How should public health policy be modified, across different spatial and temporal scales, to account for differences in the behavior and ecology of mosquitoes and humans, environmental change, and perturbations to transmission from control operations? Because it is the context in which questions like these will be answered, an improved understanding of interface between ecology and epidemiology is rich with opportunities for addressing the most important challenges in mosquito-borne pathogen prevention.

In my presentation I will explain how increasing our capacity to capture epidemiologically relevant complexity of pathogen transmission –human social interactions and mosquito ecology –will enhance disease prevention programs.Focusing on dengue, a mosquito transmitted virus, I will review the development of novel concepts in pathogen transmission dynamics that my colleagues and I are testing. I will explain why we need to expand our vision beyond the historical influence of simplifying assumptions, to explore more difficult to track variation in the rate at which mosquitoes bite different people and how human and mosquito movement influences encounters between mosquitoes and people. I will explain why exploration of mosquito ecology and behavior, mosquito and human movement, spatial heterogeneity in complex epidemiological landscapes, and the way those factors lead to key encounters between mosquitoes and hosts are among the most promising frontiers for improved mosquito-borne disease prevention.

Keywords:*medical entomology, infectious disease dynamics*

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PL4

Habitat loss and fragmentation – What happens to insect populations and species?

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Conversion of natural habitats to cultivated fields, plantations, built areas and so forth has been and continues to be the main threat to biodiversity in general and to the viability of insect populations and species in particular. For instance, forest loss in Madagascar has caused the apparent extinction of a large fraction of endemic forest-inhabiting dung beetles and undoubtedly of many other insect taxa. I discuss with examples two key concepts which describe the responses of insect populations to habitat loss and fragmentation, namely extinction threshold and extinction debt.

Habitat conversion and other global changes, especially climate change, impose new selection pressures on populations and often lead to microevolutionary changes.

I describe examples in which demographic (ecological) population dynamics and microevolutionary dynamics are closely coupled and influence each other. Microevolutionary changes may compensate for the detrimental ecological consequences of habitat loss and fragmentation, but such compensation is likely to be only partial at best and is hence unlikely to save populations and species from extinction.

When small populations become completely isolated in small or relatively small fragments of habitat, they are likely to accumulate deleterious mutations due to inbreeding and genetic drift, which may lead to permanent loss of viability and eventually to extinction. I describe an example of such mutation accumulation in the Glanville fritillary butterfly.

Keywords:*habitat loss, population extinction, genetic load and extinction*

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PL5

Insect science and poverty alleviation – tales from Africa

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International Centre of Insect Physiology and Ecology, Kenya

Insects and other arthropods are major threats to human health, livestock and agriculture in sub-Saharan Africa. Scientists at icipe (International Centre of Insect Physiology and Ecology) are developing novel solutions to control pests by manipulating insect ecology and behaviour. Malaria mosquitoes and crop pests can be pushed away from their hosts with repellant odours and pulled towards traps. Desert locusts, if prevented from communicating with each other, will not form swarms.
Tsetse flies can be lured into special traps where they are contaminated with the spores of their own fungal pathogens, which they then disseminate throughout their populations.
Controlling pests with these innovative tools, as well as promoting the commercial use of beneficial insects such as bees and silkworms, are just some of the ways that icipe is improving the health, food security and quality of life in African communities, providing practical and hands-on examples on how science can contribute to poverty alleviation in Africa.

Keywords:*Nutrition*

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PL6

Ecological Succession of Insect Populations in Bt cotton agroecosystem: a Case Analysis in China

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Chinese Academy of Agricultural Sciences, China

During the last 16 years, widespread planting of transgenic crops producing insecticidal proteins derived from the bacterium Bacillus thuringiensis (Bt) has provided highly effective control of several major insect pests of cotton and corn in the World. China started to plant Bt cotton in 1997, and extended to 3.8 million hectares in 2011 that was equivalent to 70% of the national cotton-growing area of 5.7 million hectares. Continuous annual surveys of insect pest populations in Bt cotton agroecosystem since its commercialization in China suggested that the planting of Bt cotton significantly decreases the population levels of the main target pest Helicoverpa armigera not only in cotton fields but also in other host crop fields.
As a result, the applications of broad-spectrum insecticides are largely reduced in Bt cotton fields, which consequently brings a marked increase in abundance of natural enemies that in turn provide additional biocontrol services spilling over from Bt cotton fields into neighboring crops (maize, peanut and soybean). On the other hand, due to the reduced use of insecticides, Bt cotton provides a shelter for non-target pests, mirid bugs (Heteroptera: Miridae), which have progressively increased their population levels and acquired pest status in cotton and multiple other crops.
Our work demonstrated that the growing of Bt crops can bring long-term landscape-level impacts on population evolution of insect pests and natural enemies, therefore, rational/regional wide integrated pest management strategies should be developed for sustainable use of transgenic Bt crops.

Keywords:*Bt crop, IPM, agroecosystem*

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S101M01

Systematics, Phylogeny and Zoogeography

Changing the way we describe biodiversity

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Taxonomists invest enormous effort into annotating phenomes and packaging these statements into 'descriptions'. These annotations - head black, femur longer than tibia, etc. - are usually published in analog resources (as per the current ICZN) and infrequently, if ever, used again. Frustratingly, the annotations are often reproduced de novo using inconsistent syntax, lexicons, and concepts, when a taxon is revised. Our vision centers on a new approach for describing taxa that employs a model developed in the biomedical and genomics realms: Entity-Quality statements (EQs) derived from phenotype ontologies. Ontologies represent a foundational technology for establishing shared semantics, and for capturing and computing with biological knowledge. In the EQ model, an entity term from an organism-specific ontology is associated with a quality term from a generic trait ontology, a compositional approach that has been shown to facilitate interoperability across scientific domains. Formalizing taxon descriptions in this way will avail millions of explicit data annually for researchers interested in adaptation, phenotypic plasticity, evo-devo, diagnostics, and other subdisciplines that focus on phenotypes. Moving towards a formalized phenotype-based system will have profound long-term consequences: 1) the terminology used to reference phenotypes will be unified across multiple realms of biodiversity research, thus facilitating knowledge transfer; 2) phenotype statements based on ontologies enable direct comparisons of trait variation across Life; 3) tying the phenoscape to genomics will allow broad-taxon retrieval of information relevant to the genetic control of phenotypes; 4) taxon concepts will be standardized, making diagnoses and species delimitation more meaningful.

Keywords:*biomedical ontologies, taxonomy, Entity-Quality statements*

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vSysLab: A platform for development of monographs for the digital future of taxonomy

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To maximize its impact for the future, taxonomic works should be directed not only toward the traditional reader, but also expose their results to machine-based datamining. vSysLab is a database application designed to facilitate acquisition and management of biodiversity data and to generate output for both of these 'audiences.' The application integrates nomenclatural data, literature annotations, taxonomic concepts, and character definition. The user interacts with vSysLab through standard web-based forms accessible through any browser. The mode of character and character state definition is fully compatible with entity-quality statements and, therefore, for integration of formal ontologies (such as anatomical ontologies for morphological characters). Output options for the taxon-by-character matrix include plain-text, Nexus, Lucid Interchange Files, and text implementing the developing Structure of Descriptive Data XML standard. Full taxonomic descriptions produced by the software incorporate not only character data, but also nomenclatural history, material examined summaries, links to dynamic distribution maps, as well as free text. Summaries of taxonomic and specimen data are output implementing the DarwinCore archive format. Complete documents implement the taxpub XML schema for taxonomic publications. With this semantic markup incorporated into the final document, the information is immediately accessible for datamining as well as for automated incorporation into aggregators.

Keywords:*database, cybertaxonomy, datamining, semantic markup*

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Cybertaxonomy of species rich clades using character matrices as data repositories and natural language parsing for rapid publication of species descriptions

Shaun Winterton
California State Collection of Arthropods, U.S.A.

Estimates of global biodiversity are as varied as they are enormous, but the societal need for documenting biodiversity has never been greater. The taxonomic process is as slow and careful as it is inefficient and tedious, with numerous instances of data redundancy. Natural Language Descriptions parsed from character matrices has significant promise for enhancing the process of taxonomic description to produce highly standardised taxon descriptions, coupled with the wide availability of high-resolution imaging and distributed online databases. This means that taxon descriptions can be produced rapidly in machine-readable format that is reusable and distributable (e.g. monographs, interactive keys, EOL taxon pages). A paradigm shift away from traditional handcrafted descriptions towards character matrices is required, and this 'cybertaxonomic' method holds tremendous potential for significantly increasing the output of taxonomists. Results from a recent cybertaxonomic study of species-rich genera (totalling 200+ species) of Australasian stiletto flies (Diptera: Therevidae) empirically show that hundreds of new species can be described in a timeframe far shorter than traditional taxonomic studies.

Keywords:*cybertaxonomy, Therevidae, natural language description*

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Scratchpad virtual research environments: Making your data work for you

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Scratchpads (http://scratchpads.eu/) are a flexible system for people to create their own virtual research environments supporting their biodiversity research activities. Scratchpads embed the process of data creation, archival and scholarly publication into a website that help users to work collaboratively by managing the increasingly complex range of tasks involved in carrying out research. As of March 2012 the project had over 6,000 active users across nearly 350 separate communities. This date also marked the fifth anniversary of the Scratchpad project and the release of a major new version of the software. This version combines technical enhancements that make the project more sustainable with new features that make the system more functional and easier to use. In this presentation we briefly review the first five years of operation, summarise recent developments and outline a roadmap for the next five years. With reference to entomological examples we show how people are using this tool to integrate their research activities, and using new analytical and publishing services to gain greater exposure for their data.

Keywords:*Web, database, virtual research environment, natural history, biodiversity*

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Species file software: Its past, present and the future

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The Species File Software development group provides software, tools, and support services to taxonomists in their efforts to catalogue and understand the diversity of life and to share the resulting data with the global community. The group is endowment supported with commitment to continued development and bug fixing well into the future. Currently 11 insect species files are supported with nearly 92,000 total names and 56,000 valid species names being shared with the Catalogue of Life, Global Biodiversity Information Facility, and several other data aggregators indirectly through CoL. Species Files is fully web functional, capturing and displaying nomenclature, literature, images, sound recordings, distribution, and specimen data Internet. New features include the use of Life Science Identifiers in all species files, the ability to produce private species files, tracking of nomenclatural history, Darwin Core Archives generation, and updated key design. In the future, we are building additional tools for taxonomists and offering services to the biodiversity and taxonomic community. We invite taxonomists to collaborate with us to build additional species files for their group of interest.

Keywords:*Species File Software, taxonomy, nomenclature, software tools*

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An integration of cryobanking and web-based image recognition systems for systematic studies and curation

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Contemporary systematics has incorporated multidisciplinary approaches and applied many technologies. As molecular and digital techniques become more accessible, the application of these data in assisting morphological taxonomy becomes popular. Collections of specimens are no longer confined in dry (pinned) or web (spirit-preserved); preservation of frozen materials has developed in many institutes worldwide. In Taiwan, the Cryobanking Program developed five years ago has preserved more than 1,300 species on various groups of insects from the participating academic institutions. Each specimen has reserved cryobank tissues accompanying its digital images. We have barcoded thousands of specimens in diverse groups, such moths, butterflies, longhorned beetles, stag beetles, midges, thrips, and many others. As cryobanked specimens have digital inventories and images available, we have developed web-based image recognition and automatically retrieval systems based on the uploaded images of various groups. Appropriate images based on the contour, pattern and/or color characteristics were extracted from general body background first and then went through the processes of normalization, computation, and comparison to the database. As diagnostic characters for different insect groups varied, it is necessary to develop specific algorithms for appropriate taxonomic category, such as family, superfamily, or suborder. Use of wing images in automatic recognition for giant silkmoths, an accuracy reaching 97% was shown. The current web-based recognition system not only plays a role as a user-friendly interface for a layman to learn the specimens on hand, but also serves as a useful tool for curation since not all the cryobanking materials have been identified.

Keywords:*image recognition, cryobanking, curation*

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S101M08

Systematics, Phylogeny and Zoogeography

PBI and beyond databasing and the heteropteran community

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The Plant Bug PBI (Planetary Biodiversity Inventory) database was created in 2004 primarily to facilitate revisionary taxonomic research on Miridae (Hemiptera). This talk examines how the structure, outlets, and uses of this database have changed during the past 7 years. The PBI database now embraces multiple user interfaces and serves as central resource for several other projects funded by the US National Science Foundation on systematic research of Hemiptera and other arthropods, among them several monographs of Reduviidae or assassin bugs. The PBI database has recently undergone further changes in the context of a Tri-Trophic Databasing project (TTD Digitization Thematic Collection Network) that aims on large-scale documentation of the North American fauna of predominantly phytophagous Hemiptera, their host plants, and the parasitic Hymenoptera that exploit them. The data collected during this project will be relevant for studies of climate change, plant-herbivore-parasitoid phenology, pest status and distribution, biological control, systematics, and biogeography.

Keywords:*Hemiptera, Heteroptera, database*

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S101M09

Systematics, Phylogeny and Zoogeography

From field to screen and beyond: New methods for the collection, curation, and illustration of parasitic Hymenoptera (Insecta)

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A significant portion of the 'taxonomic impediment' for parasitic Hymenoptera (Insecta) surrounds the collection of fresh material from various biogeographic regions, as well as illustrating the myriad of new species through taxonomic publications. The collection and processing of large bulk samples of arthropods is a time intensive endeavor. If the target taxa are small (1.0?5.0 mm), such as parasitic Hymenoptera, the challenge is even greater. First, we present on some techniques the we have formulated or modified that are designed to enhance the speed and efficiency by which bulk field samples can be processed. Techniques include a modified deployment system for Malaise traps, a fractionater for separating large arthropods from the small ones, and a rotating intern pool for sorting, mounting and labeling. Second, we present on some micro-photographic techniques for illustrating the species we have collected. We have developed mounting techniques that minimize the 'observer effect' while photographing small wasps, and allow us to work seemlessly between light microscopy and scanning electron microscopy. Additionally, we have some specialized LED illumination domes adapted for use with a compound microscope that help resolve very small specimens. Together, these techniques help us shorten the time lag between specimen acquisition and species description.

Keywords:*microphotography, systematics, taxonomy, description*

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S101M10

Systematics, Phylogeny and Zoogeography

Remote microscopy: A success story in Australian and New Zealand plant biosecurity

Michael Thompson
Queensland university of technology, Australia

Rapid and accurate identification of organisms is crucial to many research and applied outcomes. Diagnostics is a critical first step in determining the significance of suspected biosecurity threats posed by emergency plant pests and other invasive pests and pathogens. Remote Microscopy (RM) allows entomologists to interact in real time to identify pests and pathogens, viewing live high quality images via the internet in order to act, particularly in quarantine settings. The Cooperative Research Centre for National Plant Biosecurity's RM network has now expanded to include Thailand, Vietnam, Lao PDR, Malaysia, Indonesia, East Timor, Papua New Guinea, New Zealand and Australia with over 60 locations actively participating in identifications and using RM to educate and train each other. With the release of the new Padil (www.padil.gov.au) has come the Remote Microscope Diagnostics (RMD) portal. This dynamic, adaptable, secure, Padil image and information integrated, freely available service facilitates the "Taxonomic Triage" process, from user to diagnosis and appropriate action. The RMD portal will also include delivery of the microscope feed itself, thus eliminating many of the troublesome IT security issues that have plagued RM users in the past. The RMD portal allows users to upload information, GPS coordinates, images and video for action by a "Triage Officer" who will either identify the pest or utilize the extensive pool of taxonomic expertise found throughout the RM network and greater entomological community in Australia, New Zealand and South East Asia. The RMD portal will also allow the formation of "Triage Organisations" for application to specific lab groups or quarantine bodies, which will allow user group driven application and extensive domestic and international application. The dynamic design of the RMD portal and the extensive pool of expertise available will greatly enhance the diagnostic capacity of regional entomological communities in the form of identifications and the taxonomic triage process as well as in the realms of taxonomic training and the delivery of online training packages.

Keywords:*Remote microscopy*

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S102M01

Systematics, Phylogeny and Zoogeography

Making scents of it all: Volatile pheromones of the Cerambycidae

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Until recently, little was known of the chemical ecology of longhorned beetles, especially of the role of volatile pheromones in mate location. The last eight years have seen a dramatic increase in the number of volatile pheromones that have been identified for cerambycid species. These pheromones include several compounds that are general attractants for multiple species in multiple subfamilies. Here, we summarize the current knowledge of volatile pheromones of longhorned beetles, including information on the role of pheromones in mating systems, the diversity of pheromone structures, and possible synergistic effects of host plant volatiles with pheromones as a method of attracting longhorned beetles to traps.

Keywords:*Volatile attractant, mating behavior, longhorned beetle, longicorn, woodborer*

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Observational notes on the endophallus of Cerambycidae: Comparing everted and non-everted conditions

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As recently noticed in Cerambycidae, the structure of the endophallus of male genitalia is valuable not only for distinguishing among species, but also for phylogenetic considerations. Rubenyan (2002) introduced a technique to evert and inflate the endophallus of Cerambycidae by using fine pin and needle. It is the most valuable way for observation of the character of endophallus, but this technique is occasionally limited by the size or condition of the specimen. Furthermore, it is sometimes difficult to evert because the endophallus of Cerambycidae is sometimes very long and consists of complicated structures with large sclerites inside. Contrary to this, the method to observe the endophallus in the inflated condition without eversion is much easier, and may have a universal application for cerambycid beetles. We have adopted this way on our study of Asian Mesosini since 2007 (Yamasako & Ohbayashi 2007), and recently proposed a new observation technique (Yamasako & Ohbayashi 2011, in press). However, the structures of the endophallus in everted and non-everted states have never been compared each other. We describe the techniques and our observations on the character states in both everted and non-everted conditions.

Keywords:*Cerambycidae, endophallus, observational method*

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Cerambycidae of Bolivia (Coleoptera: Cerambycidae)

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An overview of the incredible diversity of the Cerambycidae of Bolivia is presented. This project is a joint effort between the Museo de Historia Natural "Noel Kempff Mercado" (MNKM), Santa Cruz de la Sierra, Bolivia, the Florida State Collection of Arthropods (FSCA), Gainesville, Florida, the American Coleoptera Museum (ACMT), San Antonio, Texas, and the Smithsonian Institution, Washington, DC. The project's primary objective is to conduct a survey of the Cerambycidae of Bolivia. Bolivia's large size (424,000 sq. mi.) and its geographically diverse and often mountainous terrain contributes to the challenge. There are numerous remote and virtually inaccessible areas whose cerambycid faunas remain completely unknown. Since the inception of this project, most of the collecting by participants has been in Santa Cruz Department with brief forays into Beni, Cochabamba, La Paz, and recently Tarija. As a result, although our knowledge has increased tremendously, much remains to be done.

Keywords:*Cerambycidae, Longhorned Woodboring Beetles, Bolivia, Neotropical*

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S102M04

Systematics, Phylogeny and Zoogeography

Preliminary findings into the morphology and systematics of Onciderini (Cerambycidae: Lamiinae)

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Preliminary findings from the first morphological study and cladistic analysis of the tribe Onciderini Thomson (Cerambycidae: Lamiinae) are presented. Onciderini are Nearctic and Neotropical in distribution and get their common name (the "twig girdlers") from the fact that adult females of several genera (e.g., Ecthoea, Lochmaeocles, Oncideres, Psyllotoxus) girdle branches of various host trees. Members of this tribe are known to attack more than 50 different woody plant families, including many economically and agriculturally important species such as avocado, cocoa, and coffee. The main objective of the study is to test the monophyly of the tribe, as well as relationships among the 79 genera and more than 450 species. Of particular interest is elucidating the origin of girdling behavior, as well as the potential discovery of morphological characters associated with girdling. Data presented are based on morphological dissections and illustrations of male specimens representing 20 genera. Morphological characters examined are from the head capsule, mandible, maxilla, labium, pronotum, mesonotum, prosternum, hind wings, and tegmen.

Keywords:*Cerambycidae, longhorned wood boring beetles, Neotropical, Nearctic, twig girdler, morphology*

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Cerambycidae of North Vietnam

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We present the results of an entomological expedition focussing on Cerambycidae (Coleoptera) to the provinces of Cao Bang, Thai Nguyen and Vinh Phuc in northern Vietnam, undertaken in 2011. Institutional collaborators include the United States Department of Agriculture and Smithsonian Institution, Washington DC, and the Vietnam Academy of Science and Technology's, Institute of Ecology and Biological Resource. More than 400 species of Cerambycidae were collected, of which 323 have been determined to species. One new genus and eight new species were recorded. Results are discussed, along with taxonomic and biogeographically comments on the species studied.

Keywords:*Cerambycidae, Longhorned Woodboring Beetles, Vietnam, South-east Asia.*

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The longhorned beetles of the Itatiaia National Park, Rio de Janeiro State, Brazil

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The Atlantic Rainforest stretches for about 4,000 km along the Atlantic coast of Brazil from the state of Rio Grande do Norte down to Rio Grande do Sul. It is after the Amazon and the Cerrado the third largest biome in Brazil and, according to Conservation International, it is among the top 5 biodiversity hotspots on Earth. However, at present only approximately 7,3% (94,000) of the original 1,290,692 sq. kilometers of Mata Atlantica remains in Brazil. The National Park of Itatiaia is one of the most important protected areas of the Atlantic Rainforest and from the point of view of the insects, little is known about species of Coleoptera. A survey of the Cerambycidae species recorded in the National Park of Itatiaia, Rio de Janeiro State, Brazil, is presented. The data were based on literature, field work and in the collection of the Museu Nacional, Universidade Federal do Rio de Janeiro. Five Cerambycidae subfamilies are recorded: Cerambycinae, Lamiinae, Lepturinae, Parandrinae and Prioninae. Six hundred forty-eight species are recorded to the park, representing about 30% of the Atlantic Rainforest species, sixty three new distribution records are registered.

Keywords:*Coleoptera, Cerambycidae, Atlantic Rainforest, Neotropical*

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S102M07

Systematics, Phylogeny and Zoogeography

Cerambycidae of Brazilian tropical savanna: Diversity and endemism

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The Cerrado biome is the regional name given to the Brazilian tropical savanna and is located on the large plateau that occupies the central highlands. Influenced by the 4 surrounding biomes: Atlantic Rainforest, Caatinga, Amazon and Pantanal. The fauna and flora of the Cerrado is extremely rich but little is known about insects and there is no survey concerning the Cerambycidae fauna of the Cerrado. The preliminary results shows seven hundred species distributed in five subfamilies, Cerambycinae, Lamiinae, Prioninae, Lepturinae and Parandrinae. The most diverse is Cerambycinae with 455 species of which 128 are endemic, 51 occurs also in Amazon Rainforest, 164 in common with Atlantic Rainforest and 60 are large distributed in Neotropical region.

Keywords:*Coleoptera, Cerambycidae, Cerrado*

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S102M08

Systematics, Phylogeny and Zoogeography

Phylogeny of the invasive *Agrilus planipennis* Fairmaire (emerald ash borer - EAB) and its relatives (Coleoptera: Buprestidae)

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Agrilus planipennis Fairmaire (emerald ash borer - EAB) is a highly destructive invasive wood-boring insect pest from Asia that kills healthy, non-Asian ash (Fraxinus) trees. It has invaded areas of eastern North America (U.S.A. and Canada) and Moscow, altering native landscapes and costing hundreds of millions of dollars for control and eradication. Understanding its phylogeny will allows scientists to make predictions about potential new invasive species with similar evolutionary histories and adaptations and help determine ways to manage them. A preliminary morphology-based phylogenetic analysis was undertaken to infer the sister-group relationships of Agrilus planipennis. One hundred fourteen characters were coded for 40 taxa and analyzed under parsimony. Results indicate an affinity with species in the A. cyaneoniger group and groups of species with distributions in Southeast Asia. Taxon sampling, homoplasy, and the value of larval characters is discussed.

Keywords:*Agrilus, Coleoptera, systematics, taxonomy, EAB, woodboring beetles*

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S102M09

Systematics, Phylogeny and Zoogeography

Diversity of the buprestid beetles of the Korean peninsula (Coleoptera: Buprestidae)

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This presentation provides an overview of the diversity of the buprestid beetles of the Korean peninsula. In spite of the economic importance of Buprestidae in forest and agriculture, the species diversity of these beetles in the Korean peninsula has not been well studied. In fact, no single comprehensive study has been presented on the diversity of Korean buprestids. Therefore, this presentation will provide the first review of the Korean buprestid beetles, based mainly on the results of morphological taxonomic studies. A total of 120 species in 22 genera of Buprestidae have been found in the Korean fauna; eight of these are recognized as endemic to South Korea, including six previously undescribed species. This presentation also provides the results of comparison of the species and generic diversity of Korean buprestid beetles with that of buprestid beetles in other East Asian countries.

Keywords:*Buprestidae, Coleoptera, diversity, Korea, taxonomy*

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S102M10

Systematics, Phylogeny and Zoogeography

Pseudocryptic speciation of *Chrysochroa fulgidissima* (Coleoptera: Buprestidae) with two new species from Korea, China and Vietnam

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The jewel beetle Chrysochroa fulgidissima was taxonomically reassessed based on molecular analyses of COI ans 16S rRNA gne partial sequences, and morphological approaches, excluding the subspecies Chrysochroa fulgidissima adachii. Molecular (three data sets, COI 16S, COI+16S in mt DNA) and morphological (quantitative and qualitative characters) evidence suggested that Ch. fulgidissima consists of four independent species found in seven geographical regions, namely Korea, Japan and Taiwan, China (GuangXi and Hainan) and Vietnam, and Okinawa Island. As a result of the large genetic divergences and subtle morphological differences amongst these populations, we were able to infer that the nomonotypical subspecies, Chrysochroa fulgidissima fulgidissima, is divided into three pseudocryptic species that have undergone allopartric speciation events. Thus, we propose that the subspecies Chrysochroa fulgidissima alternans should be upgraded to valid specific status. We also provide a description of two new species, Chrysochroa coreana sp. nov. from Korea, and Chrysochroa pseudofulgidissima sp. nov. from China and Vietnam.

Keywords:*Taxonomy, Chrysochroa coreana sp. nov., Chrysochroa pseudofulgidissima sp. nov., Korea*

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S103M01

Systematics, Phylogeny and Zoogeography

Marine Entomology - Then and Now

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Insects are generally thought to be absent from the sea which covers some 70% of the earth's surface. Why insects failed to conquer the largest surface area on earth has been a subject of debate for at least 100 years. In fact, insects are not only found in various marine habitats but also live in the open ocean, thousands of kilometers away from land. Why marine insects remained largely unknown was probably because few entomologists go to sea and most marine biologists are not aware of them. According to the World Register of Marine Species (WoRMS) at least two thousand insect species belonging to some 20 orders have been described. Most are members of Coleoptera, Diptera or Heteroptera. They can be found in salt marshes, mangrove swamps, rock pools, tidal ponds, beaches, bays, lagoons and the open ocean. Although adults of many species may be found on land, their larvae are often associated with algae or other marine plants that remain submerged underwater either entirely or partially during tidal rise and fall. Their life cycles are thus closely dependent or governed by tidal or lunar rhythms. The earliest paper on a marine stapylinid was published in 1783. Literature on various species appeared sporadically during the next 200 years and was widely scattered. No attempt was made to gather all the information together until 1976 when 'Marine Insects' was published (Available for free download at: escholarship.org/uc/item/1pm1485b). Research on marine insects has blossomed since then. Marine Entomology has finally come of age?

Keywords:*Marine insects, Coleoptera, Diptera, Heteroptera*

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Distribution of the ocean-skater *Halobates sericeus* and paleo-oceanography

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The oceanic water strider (or ocean-skater) Halobates sericeus Eschscholtz has disjunct distributions in the Pacific Ocean with a northern and a southern population widely separated by an equatorial zone. It is sensitive to sea surface conditions. Consequently, its distribution and population structure can provide insight into environmental changes on the ocean surface on both recent and historical scales. We assessed the genetic diversity and population structure of H. sericeus in the Pacific Ocean using one mitochondrial (COI) and two nuclear (EF1α, ITS-1) gene markers. These genetic markers indicated that the northern and southern populations are evolutionarily distinct with limited gene flow, and that these populations separated 20 to 50 thousand years ago. This implies that physical conditions or biotic interactions on the surface of the Pacific Ocean have provided significant barriers to gene flow since the late Pleistocene or earlier, creating biotic stability over large geographical and temporal scales in spite of a long history of global climate change.

Keywords:*Divergence time, Halobates, Pacific Ocean, Population structure*

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Temperature tolerance of oceanic sea skaters, *Halobates* (Heteroptera: Gerridae), and ocean dynamics

Tetsuo Harada[1], Shiho Takenaka[2], Takero SEKIMOTO[3], Yuki OSUMI[4], Takao INOUE[5], Mitsuru Nakajyo[6], Chihiro KATAGIRI[7]
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This study was performed to clarify how weather and ocean dynamics affect resistance to temperature change in the oceanic sea skaters, Halobates. Heat coma temperature (HCT) was measured mainly for the adultsof threeHalobates species collected from a fixed sampling location (12°N, 135°E) and other locations (0ºN-8N, 12ºN-17ºN,130ºE-140ºE) in the Western tropical Pacific Ocean,and also from 13locationsin the eastern area of the India Ocean (08°N- 6°S, 86º'E-76ºE). Halobatessericeus(distributed in the wide latitude zone of 5ºN-40ºN)has shown higher HCT (35.6 ºCon average) than H. germanus (distributed zone of 0ºN-35ºN)(32.9 ºC)and H. micans (distributed mainly around the equator) (31.6ºC) (P=0.035) in a sampling area of the Western Pacific Ocean. The average HCT of individuals collected from the Pacific Ocean was significantly higher during the first half (10 days) of the sampling period at the fixed sampling point than during the second half, at 35.0ºC and 34.0ºC, respectively. The reduction in heat tolerance during the second half may have been caused by a decrease in air temperature due to rain fall that occurred around the sampling point during the arrival of Typhoon No. 6. Adultsof H. micans and H. germanuscollected from the area within 75ºE-95ºE, 5ºS-10ºS of the Indian Ocean, showed extremely high HCTs of 37º-40ºC on average.The exceptionally strong heat tolerance may be related to the fact that this area shows the largest intra-seasonal air temperature variation among all oceans on the globe.

Keywords:*Currents, Distribution, Heat tolerance, Oceanic dynamics, Oceanic Halobates,*

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Ecology of three endangered sea skaters (Heteroptera: Gerridae) in Japan

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Halobates matsumurai, H. japonicus and Asclepios shiranui are the only coastal sea skaters known in Japan. For the past several decades they have become rare in most localities and have now been designated as endangered. Their present distribution ranges are more limited compared to those in the 1950's or earlier. They are found in quiet waters along jagged coastlines without overdevelopment or pollution. Among the three, the habitat of A. shiranui is most restricted, known only in the coasts of Tsushima Island and northwestern Kyushu Is. H. matsumurai is found along the coasts of Tsushima Is., northwestern Kyushu Is. and southwestern Honshu Is. On the other hand, the records of H. japonicus show it scattered over wide ranges, extending more than 1500 km from the southwestern coast of Honshu Is. to the Nansei Islands. A. shiranui and H. matsumurai often co-occur in small coves or inner bays but H. japonicus has never yet been found to co-occur with either A. shiranui or H. matsumurai. Year-round studies on life cycles were carried out on H. matsumurai and A. shiranui in the northwestern coast of Kyushu Is. Both appeared to have more than one generation a year and to overwinter in the egg stage. Eggs of H. matsumurai were laid on natural sandstones and man-made sandstone walls along the shore, mostly above the average sea level. The eggs had very hard shells, presumably adaptive to protect them from desiccation, solar radiation and wave action, especially during the overwintering period.

Keywords:*Marine insects, Halobates, Asclepios, endangered species, geographical distribution, life cycle*

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Ecology, distribution and molecular phylogeny of the marine midge *Pontomyia* (Diptera: Chironomidae)

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Pontomyia (Diptera: Chironomidae) is a genus of exclusively marine and flightless midges with one of the shortest known adult life spans (< 3 hours). Larval stages are spent entirely underwater among submerged vegetation or algal turf. Males have modified wings to propel them over water while females are wingless, almost completely legless and depend on the males to find and mate with them on the sea surface. Males die shortly after mating and females die after laying eggs which are encased in a gelatinous coil. Four species have been described: P. natans, P. cottoni, P. oceana, and P. pacifica. Pontomyia cottoni is endemic to Australia while the remaining three species are widely distributed along island shores in the Indo-Pacific. One species from the Atlantic remain to be described since no adult males have yet to be collected. Results from a four-gene phylogenetic reconstruction showed that P. natans, P. oceana and P. pacifica are each well-supported clades, with P. natans as sister to P. oceana + P. pacifica. The commonest and most widespread species, P. natans has been collected from Samoa, Japan, Fiji, Malaysia, Marshall Islands, Queensland Australia, and Taiwan in the Pacific, and Maldives and Christmas Island in the Indian Ocean, locations that are thousands of kilometers apart. Males from different locations are morphologically indistinguishable, but genetic distances based on mitochondrial COI are extraordinarily large within this species (up to 15%), indicating possible cryptic speciation. Mechanisms for cryptic speciation in these unique marine midges remain to be discovered

Keywords:*Pontomyia, marine midges, distribuion, phylogeny, ecology*

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Live with the rhythm of the tides: Evolution of local adaptations in adult emergence time of the marine midge *Clunio marinus* (Diptera: Chironomidae)

Tobias S. Kaiser
Max F Perutz Laboratories, Austria

Marine midges of the genus Clunio HALIDAY 1855 are found on many coasts worldwide. All species of the genus that were studied in detail display diurnal and lunar rhythms of adult emergence, which enable them to synchronize reproduction to the most suitable tides. As the pattern, amplitude and timing of the tides differs along the coast, Clunio species with different geographic distribution as well as different populations of wide-spread Clunio species require specific timing adaptations to the local tides. Phylogenetic analysis of the European species of the genus Clunio revealed that there is both convergent and divergent evolution. Genetically closely related species, such as Clunio marinus and Clunio balticus, have divergent timing adaptations due to the different ecological requirements of their habitat. In turn, genetically divergent species, such as Clunio mediterraneus and Clunio ponticus, have similar timing phenotypes due to identical ecological niches. The species Clunio marinus, being distributed along the European Atlantic Coast from Spain to Iceland, comprises a variety of locally adapted timing races. A population genetic survey revealed that likely all of these timing races are derived from the same ancestor and evolved within less than 20,000 years, underpinning the enormous evolutionary flexibility of Clunio's timing traits. Crossing experiments with Clunio marinus identified some of the genomic loci that control the timing traits and showed that lunar and diurnal timing are genetically not independent. The ongoing Clunio marinus genome project will soon enable the study of these timing traits and their evolution at a new level.

Keywords:*biological clock, lunar rhythm, circadian rhythm, evolutionary genetics, population genetics, quantitative trait locus, molecular systematics*

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A marine insect that counts - Mechanism of Circa-semilunar emergence rhythm of *Pontomyia oceana* (Diptera: Chironomidae)

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Lunar and semilunar cycles of biological activities are common in marine and even some terrestrial organisms (Endres & Schad, 2002; Naylor, 2010), of which some are actually controlled by endogenous oscillations that are in turn entrained by environmental cues (Hauenschild, 1960; Neumann, 1966; Saigusa, 1980; Reid & Naylor, 1985; Luning et al., 2008). How the periods (e.g., ~15 or ~30 d) of such internal rhythms are maintained in the absence of external entraining factors has been a challenging question (Neumann & Heimbach, 1985). This paper reports a case supporting the frequency demultiplication hypothesis, in which the internal circasemilunar emergence rhythm of an intertidal midge, Pontomyia oceana, is based on counting the cycles of endogenous circadian rhythms.

Keywords:*marine midge, semilunar, emergence, endogenous, Chironomidae, biological clock*

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S103M08

Systematics, Phylogeny and Zoogeography

Zoogeography of coastal marine Staphylinidae (Coleoptera)

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Staphylinidae (Coleoptera) are currently the insect family with the most described species, over 55,000, and yet perhaps as many as 80% of the world's staphylinid species are undescribed. Fewer than 400 staphylinid species have yet been determined to be obligate inhabitants of seashores of the world. Evolution of specialization to coastal habits is polyphyletic among coastal Staphylinidae, occurring in eight subfamilies. Aleocharinae are the most species rich of the eight subfamilies with coastal representatives. The Pacific Ocean has many more species of coastal staphylinids than do the other oceans. The United States has more species of coastal staphylinids than do eight other countries. Some species (Group A) belong to genera having mainly non-coastal species, whereas some (Group B) belong to genera in which all species are coastal. In Group B, 40 genera occur only on the shores of the Pacific Ocean, and 10 genera only on the shores of the world's other oceans, suggesting that the Pacific Ocean is the main cradle of evolution of specialist coastal Staphylinidae. In Group A, about as many species are known from the other oceans as from the Pacific as might be expected when evolution at the species level is more recent than at the generic level. It is appropriate that this symposium is held in South Korea, a Pacific-rim country rich in coastal Staphylinidae.

Keywords:*Staphylinidae*

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Phylogeny of the marine littoral genus *Cafius* (Coleoptera: Staphylinidae)

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A phylogenetic analysis of the marine littoral genus Cafius Stephens is presented based on molecular characters. The data set comprised partial mitochondrial COI (910 bp), COII (369 bp), 12S rDNA (351-354 bp), 16S rDNA (505-509 bp) and nearly complete sequences of 18S rDNA (1814-1830 bp) for 37 species. Twenty-seven Cafius species, representing five of six subgenera, two Remus Holme species, three Phucobius Sharp species, monotypic Thinocafius Steel and four outgroups were included. The sequences were analysed simultaneously by parsimony analysis in Tree Analysis Using New Technology (TNT) with traditional manual alignment, direct optimization (DO) in the program POY4 under a variety of gap costs and partitioned Bayesian analysis for the combined data. The genus Cafius and nearly all of its subgenera were not supported as being monophyletic. Instead, all analyses (parsimony trees, DO tree under equal weighting and Bayesian tree) showed monophyly of Cafius + Phucobius + Remus + Thinocafius (clade Z) and all seven nested clades (A-G). However, the phylogenetic relationships among clades A?G differed among the analyses. The genus Phucobius was recovered as a monophyletic group within Cafius. The genus Remus was not monophyletic but formed a clade with C. rufescens Sharp and C. rufifrons Bierig within Cafius. The genus Thinocafius formed a clade with C. caviceps Broun, C. litoreus (Broun) and C. quadriimpressus (White) within Cafius. We propose new concepts for the genus Cafius and its related genera, and the seven nested clades.

Keywords:*Molecular phylogeny, marine littoral, Cafius, taxonomic implication*

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Molecular phylogeny and coastal habitat evolution of the genus *Aleochara* (Coleoptera: Staphylinidae)

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A revised molecular phylogeny of the genus Aleochara Gravenhorst is presented. The data set comprised partial mitochondrial COI (1373 bp), COII (577 bp), and complete sequences of tRNA leucine (71 bp) between them, for 56 Aleochara and eight outgroups. We added eight coastal species with 15 populations (Aleochara fucicola Sharp, A. littoralis (Maklin), A. nubis Assing, A. puetzi Assing, A. squalithorax Sharp, A. sulcicollis Mannerheim, A. trisulcata Weise, and A. zerchei Assing), which were not included in the previous analysis. The sequence data were analyzed by Maximum Likelihood, Bayesian and parsimony methods. The monophyly of the genus Aleochara, curtula clade, bilineata clade and clades A to D containing the coastal species were strongly supported by all of three analyses. Based on the phylogenies, we hypothesize that multiple origins of seashore habitat have occurred throughout the genus Aleochara.

Keywords:*Aleocharinae, Molecular phylogeny*

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Histeridae, Salpingidae and Hydrophilidae (Coleoptera) on seashore of Japan

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Japanese coastline is the sixth longest in the world being about 29,751 km. Japan is also a very long country from north to south, the northernmost point is at 45˚33' N and the southernmost is at 20˚25'N, its distance is same from Milano, Europe to Sahara Desert, in Africa. Animal living on the Japanese seashore vary enomously and show the Palaearctic to Oriental zoogeograpical elements. Fauna of the littoral beetles is also very rich, especially the families Staphylinidae, Hydrophilidae, Histeridae, Agyrtidae, Scarabaeidae, Elateridae, Tenebrionidae, Salpingidae and Curculionidae. Topics for this presentation as follows: (1) On the general information of the Japanese littoral beetles. (2) On the species of the genera Hypocaccus, Eopachylopus and Hypocacculus (Histeridae) and their distributions. (3) On the species of the genus Aegialites (Salpingidae) and their distributions. (4) On the species of the genus Cercyon (Hydrophilidea) and their distributions, habitat and phylogeny. Additionally, the damage of the coastline and animal living on seashore by Tsunami, 11th March 2011, in Tohoku area, Japan is reported.

Keywords:*littoral, beetle, Tsunami, costline*

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Concluding remarks

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This symposium on marine insects is coming to an end and I would like to thank very much the speakers and organizers for giving me the floor to be the last speaker and to make the closing remarks. It is a special honor for me and I really appreciate it. I consider this half-day meeting as a highly productive and informative gathering because of the excellent scientific quality of all the presentations. Taxonomically, our symposium could be divided into three parts, three on Gerridae (Heteroptera), three on Chironomidae (Diptera) and four on Coleoptera. It may be useful to point out that this is the third symposium on marine insects during an ICE. The first occurred in Washington D.C. in 1976 and the next was held in Florence, Italy in 1996. I will be trying to give some personal views on what seemed to me the most important. As a taxonomist of the beetle family Staphylinidae, I can say that I learned a lot about marine insects. I hope that you learned a lot as well. I wish that this symposium will serve as a stepping-stone toward future research programs and networks on marine insects.

Keywords:*marine insects, ecology, evolution, phylogeny, biogeography*

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S104M01

Systematics, Phylogeny and Zoogeography

Elucidating Lepidoptera evolution: A tale of two approaches

Niels Peder Kristensen
Natural History Museum of Denmark, Denmark

The grand picture of lepidopteran evolution has long been drawn exclusively on the basis of morphological findings. It showed a tentatively resolved initial sequence of origins of non-ditrysian lineages followed by a radiation at the basis of the Ditrysia, which is only very partially resolved. The molecular approach to Lepidoptera systematizing, with analyses of sizable gene and taxon sets, have led to considerable progress in refining family-/superfamily-level classification, but few relationships above superfamily level are yet robustly supported. Expectedly forthcoming massive novel genomic data may enable significantly better resolution of this ditrysian 'backbone', but if many ditrysian superfamilies indeed originated within short time spans, a number of internodes in the tree may remain retrievable with difficulty, if at all. The role of the morphological approach to evolutionary research on Lepidoptera will change. Morphological characters will continue to assist in choosing between alternative solutions emerging from molecular analyses, and/or may highlight need for additional investigation in cases where results from molecular analyses appear conclusive when viewed in isolation. But the role of morphological findings will increasingly be providing data, which, when mapped on molecule-generated cladograms, add to the interest of the latter. Glossatan Lepidoptera may seem to be overall structurally homogeneous, but much structural modifications actually did take place during their evolution. Several long-standing questions about "where?", "how many times independently?", and "in how many recognizable steps?" notable structural innovations evolved still remain unanswered.

Keywords:*Evolution, molecules, morphology*

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S104M02

Systematics, Phylogeny and Zoogeography

From soil to tree: the ground plan of the lepidopteran larval trunk, and notable early deviations

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Natural History Museum of Denmark, Denmark

The 'typical' neolepidopteran caterpillar represents the larval type of more than 99% of the Lepidoptera. The 'typical caterpillar' with crotchet bearing abdominal prolegs has however evolved from a very different soil dwelling larvae type without prolegs. The study of larvae of the non-Neolepidoptera including Micropterix, Agathiphaga, Heterobathmia, Eriocrania and Acanthopteroctetes is used to discuss the evolution and morphology of the 'typical caterpillar'. The Micropterix and Agathiphaga have larval with trunk a trunk morphology too derived to discuss the characteristics of the larval ground plan, they have important life histories that reflect a succession form a soil dwelling organism to that of a more arboreal free living organism as seen in the neolepidopterans. The leaf mining life style and the larval morphology of Eriocrania and Acanthopteroctetes and especially Heterobathmia appear to be informative with regards to the lepidopteran larval ground plan and the trunk morphology of the 'typical caterpillars'. The trunk morphology of the three non-neolepidopterans is therefore presented in order to discuss three key points; 1) the Evolution of the autapomorphic apodemeless trunk musculature, 2) the secondary locomotory structures (calli) of the leaf mining larvae and 3) the calli as possible structural predecessor to the abdominal proleg

Keywords:*Basal Lepidoptera, Larvae, Morphology*

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S104M03

Systematics, Phylogeny and Zoogeography

More genes, more taxa, more questions? Inching towards a molecular phylogeny of Lepidoptera

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While detailed morphology-based studies provide well supported hypotheses for early splits into several (now) relatively species-poor families, relationships between families and superfamilies of the Ditrysia, which comprise 98% of the approximately 157,000 named lepidopteran species, are still rather poorly understood. Recent progress in molecular phylogenetics of Lepidoptera resulted in numerous new hypotheses, several of which are reflected in a publication (van Nieukerken et al. 2011: Zootaxa 3148) that provides a revised higher classification of the order. This presentation outlines the changes and compares the evidence in support of these changes with unpublished results from a much larger molecular study, the 'Assembling the Tree of Life Lepidoptera' project (http://www.leptree.net). These new results are based on up to 19 nuclear, protein-coding genes (18.9kbp) for 847 species that represent almost all lepidopteran families and subfamilies. The broad taxon and gene sampling provides numerous well supported phylogenetic hypotheses within families and superfamilies, many of which recover or complement existing hypotheses. In contrast, the 'backbone phylogeny' of Ditrysia, i.e., relationships between superfamilies, has exceedingly short branches and remains only partly resolved.

Keywords:*molecular phylogeny classification Lepidoptera Ditrysia*

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S104M04

Systematics, Phylogeny and Zoogeography

On the phylogenetic affinities of some enigmatic Lepidoptera

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The phylogeny of the order Lepidoptera has recently been under several molecular investigations. While the monophyly of many lepidopteran superfamilies and lower ranks seems well-established, the deepest nodes remain frequently ambiguous. Similarly, the phylogenetic affinities of several rare and/or enigmatic taxa remain to be scrutinized. We have added several taxa of particular interest to the previously published 350 species dataset that comprehensively represents the lepidopteran diversity. The added taxa include Siloscinae, Tanaoctena, Copromorpha, Callizygaena, Meharia (2 spp.), Prodidactis and an unknown, recently discovered and unnamed taxon not associated with any superfamily. We were able to associate, at least roughly, these taxa with other lepidopteran taxa, some with high, others with lower confidence. Even so, the interrelationships of deeper nodes remain uncertain and require further attention.

Keywords:*Lepidoptera, Molecular phylogenetics, Systematics*

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S104M05

Systematics, Phylogeny and Zoogeography

Getting a sense of time for massive radiations in Lepidoptera

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University of Turku, Finland

The diversity of life on Earth is enormous and our task of documenting it is still underway. One important part of this documentation is understanding the generation and maintenance of biodiversity over evolutionary time frames. When have major divergences happened? How have these divergences happened? I will be investigating these questions using the megadiverse clade of Lepidoptera (butterflies and moths) as my model group. In this talk, I will review what we know about the evolutionary history of Lepidoptera and point to the directions we are going in the future to understand why there are at least 150,000 extant species in all the world's habitats.

Keywords:*Diversification, Lepidoptera, Jurassic, Cretaceous*

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Progress toward fossil and molecular dating for the Lepidoptera Tree of Life

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Recent remarkable progresses in molecular clock methods make it feasible to estimate divergence times for phylogenetic branch points, and such estimates have become routine in evolutionary studies. However, reliable molecular dating is strongly dependent on having multiple fossils that are securely identified and of sufficient age. The availability of such fossils is strongly taxon-dependent. Among the five largest insect orders, only the Lepidoptera have a fossil record less rich than what its modern diversity would suggest (Labandeira & Sepkoski, 2003). This depauperate record and the paucity of explicitly synapomorphy-based identifications of lepidopteran fossils have hindered rigorous tests of evolutionary hypotheses for Lepidoptera. Several empirical studies have highlighted the critical effects of the number and temporal distribution of fossil calibrations, interpretation of the relationships between fossil and extant taxa, and how fossil constraints are treated in molecular dating analyses. The Lepidoptera Tree of Life Consortium has completed a catalog of all known lepidopteran fossils, and is completing an estimate of phylogeny across the Lepidoptera based on 19 nuclear genes. Together these tools will make it possible to explore how fossil calibrations affect divergence time estimates for lepidopteran lineages, and hence to develop reliable molecular dating schemes for the Lepidoptera. We provide an updated overview of the lepidopteran fossil record and identify a suite of promising fossil calibrations for molecular dating analyses of Lepidoptera.

Keywords:*Lepidoptera, evolution, paleoentomology, fossil record, molecular dating*

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Hepialoidea revisited in the light of the discovery of a new higher-level taxon

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Hepialoidea comprise only a few hundred species in approximately 60 genera, however, the superfamily is of considerable evolutionary and zoogeographical importance. It comprises the majority of the sister lineage of Heteroneura and, apart from the almost globally distributed Hepialidae s. str. and the Holarctic genus Gazoryctra, the major lineages within the subfamily have limited known distributions restricted to Australia, southern Asia, sub-Saharan Africa and the Neotropics, with each lineage usually being restricted to a limited area within a region. The superfamily thus remains crucial for understanding the early evolution and zoogeography of the whole Lepidoptera. Unfortunately the higher-level relationships of the 12 principal lineages of Hepialoidea are very poorly understood, and studies into the superfamily's evolutionary biology are therefore at the present little more than educated guesses. This lack of understanding is partly due to the scarcity of specimens in collections and partly due to difficulties in establishing which taxon is the sister group of the remaining superfamily. Here we report the discovery of a new, higher-level taxon in Hepialoidea together with re-examination of the adult morphology of key taxa, and discuss the implications for understanding the phylogenetic relationships and classification of the superfamily.

Keywords:*Hepialoidea*

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Phylogeny of the lower Heteroneura: A bunch of tiny leafminers, gallers and flower feeders

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With just over 1.1% of all lepidopteran species, the few monotrysian families constitute a very small portion of the Heteroneura, largely formed by the Ditrysia. However, knowledge of these basal clades is essential to understand how Ditrysia could arise and flourish. Recent molecular studies never support a monophyletic Monotrysia, and the 19 gene analysis (Regier et al., in preparation) reveals four monophyletic clades: Nepticuloidea, Andesianidae plus Adeloidea, the Australian Palaephatidae plus Tischeriidae and the south American Palaephatus as sistergroup to Ditrysia. Monophyly of Australian palaephatids with Tischeriidae is a novel finding. Within Nepticulidae the division in the Australian Pectinivalvinae and the rest is not supported in the 19 gene analysis nor in a 3 gene analysis with more taxa. Rather, Trifurculini are sistergroup to a clade comprising both Pectinivalvinae and Nepticulini. Within Adeloidea, the genus Tridentaforma forms a separate clade, not in the Prodoxidae, and Adelidae are not monophyletic, unless Heliozelidae are incorporated. Although leafmining occurs in many groups, the phylogeny does not rule out the possibility of multiple origins of leafmining from other ways of endophytic feeding. In Heliozelidae we report an undescribed high diversity both in North America and Australia. An initial analysis shows that Vitaceae might be a possible ancestral host family. Australia has probably as many species as are known in the rest of the world, with potentially several new genera and possibly a novel life history involving flower or seed mining. Several known North American species are part of a complex of cryptic species.

Keywords:*Nepticuloidea, Adeloidea, Palaephatidae, Molecular Phylogeny*

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Acanthopteroctetidae and their importance for understanding the early evolutionary history of Lepidoptera

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University of Copenhagen, Denmark

Based on comparative morphology, Acanthoptertoctetidae are believed to be one of the groups that arose in the earliest evolution of Lepidoptera, successively splitting off after four other lineages: Micropterigidae, Agathiphagidae, Heterobathmiidae, and Eriocraniidae. Hence, this family is the putative sister group to all other Lepidoptera, a clade containing about 99.9% of the order's species richness that includes all but one of the 'tongue-moths' or Glossata (excluding Eriocraniidae, the earliest glossatan lineage). Despite such great species richness, this large clade is morphologically uniform compared with the earliest lineages of Lepidoptera. Studying the morphology of Acanthopteroctetidae in the context of a new multi-locus molecular phylogeny of these ancient lepidopteran lineages will help us determine how the structural features of Acanthopteroctetidae relate to the lepidopeteran and glossatan ground-plans. I will discuss the implications of the new molecular results with special reference to the morphology of the acantopteroctetid adults.

Keywords:*Glossata, molecular phylogeny, morphology*

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S104M10

Systematics, Phylogeny and Zoogeography

20 million years of evolution in Hawaii's most diverse genus, *Hyposmocoma* (Cosmopteridídae)

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Hyposmocoma is an endemic Hawaiian radiation with perhaps 1,000 species, most of which have not been described. The caterpillars all carry silk cases which show spectacular variation across lineages and which are frequently diagnostic. The larvae also vary widely in their ecologies, and while most are scavengers or herbivores, some represent the only case of snail-eating Lepidoptera. Other lineages are amphibious, spending weeks totally submerged or on dry ground, representing unprecedented tolerances for aquatic/terrestrial transitions. The relationships between over 12 of these divergent lineages are strongly supported by multi-gene analyses under both Maximum likelihood and Bayesian models. Results suggest remarkable complexity in the patterns of speciation and generation of evolutionary novelty across Hyposmocoma. Even taxa on the ancient atolls of the Northwest Hawaiian Islands represent a mix of basal-branching and more derived lineages. Further, molecular data suggests that each volcano on each island supports a unique fauna of Hyposmocoma species, arguably the finest-scale example of speciation across the archipelago. These patterns suggest that Hyposmocoma are effective dispersers over long time periods, but not over the relatively short periods needed to prevent localized speciation. The extremely restricted distributions of Hyposmocoma species suggest they would be good models for conservation planning, and that they may have already suffered widespread extinction of species due to anthropogenic perturbations of the islands.

Keywords:*Lepidoptera, adaptive radiation, carnivory, island biogeography*

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S104M11

Systematics, Phylogeny and Zoogeography

Phylogenetic relationships of Gelechiidae (Lepidoptera)

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The Gelechiidae (Lepidoptera) are one of the largest families in microlepidoptera with about 4,530 species in 507 genera in four subfamilies in the world (Hodges, 1999). The higher classification of subfamilies and tribes within the Gelechiidae has been controversial. Karsholt and Razowski (1996) recognized three subfamilies and seven tribes in this family: Gelechiinae (Apatetrini, Anomologini, Teleiodini, Gelechiini, Gnorimoschemini, Anacampsini, and Chelariini), Pexicopiinae, and Dichomeridinae. Hodges (1999) recognized four subfamilies (Physoptilinae, Gelechiinae, Pexicopiinae, and Dichomeridinae) without considering tribes. Recently, Ponomarenko (2006) used male genital musculature and traditional morphological characters to propose five subfamilies and twelve tribes: Physoptilinae, Anomologinae (Anomologini, Apatetrini, Aristoteliini, Pexicopiini), Gelechiinae (Gnorimoschemini, Gelechiini, Litini), Anacampsinae (Anacampsini, Brachmiini), and Dichomeridinae (Dichomeridini, Chelariini, Anarsiini). This study provides the phylogenetic relationships of higher taxa in family Gelechiidae. Additionally phylogenetic relationships of Holarctic Litini (formerly Teleiodini) are postulated with the analysis of evolution of host plant preferences and biogeography.

Keywords:*Gelechiidae, Litini*

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Phylogenetic relationships of Tortricidae - the scene from root to fruit

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A recent molecular phylogeny of Tortricidae is reviewed and compared with historical hypotheses regarding phylogenetic relationships based on various morphological characters. The molecular phylogeny supports Bactrini + Endothenini + Olethreutini as the sister group to Enarmoniini + Eucosmini + Grapholitini, whereas the Horak-Brown hypothesis based on morphology proposed a paraphyletic relationship of Olethreutini to the latter tribes. The Horak-Brown hypothesis that Eucosmini is paraphyletic relative to Grapholitini was not supported by the molecular phylogeny, but the latter may have been biased by the low number and choice of genera sampled. Phylogenetic relationships of the tribes of Olethreutinae are reanalyzed by using novel morphological characters of de-scaled moths, e.g., the endoskeleton, appendages, and patterns of scale sockets, combined with traditional characters of wing venation and shape, male sex scales, forewing pattern, and male and female genitalia.

Keywords:*Tortricidae, Olethreutinae*

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S104M13

Systematics, Phylogeny and Zoogeography

Non-synonymous changes and increased gene sampling improves phylogenetic estimates for higher-level groups within leaf-mining moths and relatives (Lepidoptera: Gracillariidae)

Akito Yuji Kawahara[1], Issei Ohshima[2], Atsushi Kawakita[3], Jerome Regier[4], Charles Mitter[5], Michael Cummings[6], Donald Davis[7], David Wagner[8], Jurate De Prins[9], Carlos Lopez-Vaamonde[10]

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Researchers conducting molecular phylogenetic studies are frequently faced with the decision of what to do when weak branch support is obtained for key nodes of importance. As one solution, the researcher may choose to sequence additional orthologous genes of appropriate evolutionary rate for the taxa in the study. However, generating large, complete data matrices can become increasingly difficult as the number of characters increases. A few empirical studies have shown that augmenting genes even for a subset of taxa can improve branch support. We target Gracillariidae, a Cretaceous-age (~100 Ma) group of leaf-mining moths to test whether the strategy of adding genes for a subset of taxa can improve branch support for deep nodes. We initially sequenced ten genes (8,418 bp) for 57 taxa that represent the major lineages of Gracillariidae plus outgroups. After finding that many deep divergences remained weakly supported, we sequenced eleven additional genes (6,375 bp) for a 27-taxon subset. We then compared results from different data sets to assess whether one sampling design can be favored over another. Partially or fully augmenting a data set with more characters increased bootstrap support for particular deep nodes, and this increase was dramatic when non-synonymous changes were analyzed alone. The addition of sites that have low levels of saturation and compositional heterogeneity greatly improved results. We also apply new transcriptome data and examine the effects of adding these data to infer the phylogeny of Gracillariidae.

Keywords:*Gracillarioidea, Leaf-mining moths, phylogeny, sampling design, Yponomeutoidea*

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S104M14

Systematics, Phylogeny and Zoogeography

The evolutional signal obtained from Afrotropical taxa might significantly change our traditional understanding of the global diversity pattern and phylogeny of Lepidoptera

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Royal Museum for Central Africa, Belgium

Our dataset contains 33,541 species group names of moths from sub-Saharan Africa and 44,521 distribution records. With this dataset available online as a searchable website (www.afromoths.net), we aim to disseminate the obtained knowledge and to draw the main guidelines in providing a framework for the understanding of the evolutionary pattern of this model insect group. We stress herewith that the Lepidoptera of sub-Saharan Africa play a prominent role in systematic/biodiversity surveys as indicators of habitat disturbance and biodiversity hotspots. They constitute a mega-diverse group of insects with at the latest count 157,424 recognized species and an additional estimated 230,000 species still to be discovered, mostly in tropical areas. The least known Lepidoptera that include the highest number of undescribed species are the micro-moths, a paraphyletic group ignored by many lepidopterists. Over recent decades, molecular methods have complemented the traditional comparative morphology and taxonomic classification of micro-moths. Furthermore, we studied the taxonomic history of the groups which were treated controversially and in need of explanation, after their primitive position in the phylogenetic clades was established. Field work was carried out for more than 10 years in fast changing African biotopes which are still suitable for primitive moths. In recent years we described more than fifty new species of primitive moths, belonging to the superfamilies Gracillarioidea and Gelechioidea, collected in various parts of sub-Saharan Africa but mainly in primary rain forests of Central Africa. Our future intentions are to clarify the major paths of the geographical dispersal of Afrotropical moth species.

Keywords:*Afromoths*

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S104M15

Systematics, Phylogeny and Zoogeography

Evolution of pollination behavior and mutualism in *Epicephala* (Gracillariidae)

Atsushi Kawakita
Kyoto University, Japan

Four insect lineages have independently evolved to pollinate their host plants in order to secure food for their seed-consuming larvae. They are the fig wasps, the yucca moths, the senita moth and the Epicephala moths. This intricate behavior has lead to the evolution of remarkable mutualisms between the insect and the plant, where both partners depend totally on each other for reproduction. Epicephala moths are specialized seed feeders of plants in Phyllanthaceae. Adult female Epicephala moths uses their proboscises to actively collect pollen on male flowers and pollinate female flowers, and lay eggs in the flower that she just pollinated. Emerging larvae feed on a fraction of the developing seeds to complete larval development, leaving the rest intact. Extensive observation in the tropics has revealed that this mutualism is widespread globally, with estimated 600 Phyllanthaceae species each pollinated by a species-specific Epicephala. Phylogenetic analysis indicates that the pollination behavior evolved once in Epicephala about 25 Ma. Subsequent diversification of the Epicephala occurred on six distinct lineages of Phyllanthaceae, suggesting that the mutualism evolved multiple times independently in Phyllanthaceae. Some Epicephala species secondarily evolved ability to induce flower galls without needing to pollinate, thus reversing the association from mutualism to pure parasitism.

Keywords:*Epicephala, Phyllanthaceae, mutualism, pollination, phylogeny, Gracillariidae*

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S104M16

Systematics, Phylogeny and Zoogeography

The genetics of host shifting in a leaf-mining moth *Acrocercops transecta* (Gracillariidae)

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Adaptation to a new environment often requires the evolution of multiple traits, and hybridization between ecologically divergent taxa could produce maladaptive phenotype combinations to result in isolation barriers. In phytophagous insects a precise combination of performance and preference for particular host plants is crucial, and divergence in host associations is considered as a first step of speciation. However, very little is known about the genetic bases of performance and preference traits. Here I show that opposite directions of dominance in performance and preference traits play a key role in host race maintenance in the leaf-mining moth Acrocercops transecta. In this species performance on Juglans is dominant over that on Lyonia, but preference for Lyonia is dominant over that for Juglans. This indicates the genetic components that introgressed from the Lyonia race being removed from the Juglans race, while gene flow will occur from the Lyonia race to the Juglans race. Thus, the different mode of inheritance between performance and preference itself function as an isolating barrier between populations with different host associations. Linkage and QTL analyses revealed that only a restricted region of a single autosome was responsible for the differences in larval performance between the two host races. This indicates that host shifting from Juglans to Lyonia in A. transecta involved changes in a few genes with large effect, suggesting that a few genetic changes in larval-performance loci promoted the successful host shifting.

Keywords:*Larval performance, leaf miner, linkage analysis, oviposition preference, QTL mapping*

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Ever since Kyrki: A molecular phylogeny for Yponomeutoidea (Lepidoptera, Ditrysia) with new insights into their evolutionary history

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Yponomeutoidea represent one of the major radiations in the basal ditrysian Lepidoptera. Yponomeutoidea are especially important for tracing the early evolutionary history of Lepidoptera-plant interactions because they are one of the earliest groups to evolve external feeding (Powell et al., 1998) and to extensively colonize herbs as well as shrubs and trees (Grimaldi and Engel, 2005). Despite its importance to tracing the early evolution of Lepidoptera, the superfamily Yponomeutoidea has been neglected by systematists, and its biodiversity and phylogeny remain poorly understood. Due to the lack of phylogenetic approaches, several researchers have suggested different definitions of Yponomeutoidea which are still in contention. To improve the systematic resolution of Yponomeutoidea, phylogenetic analyses based on 8 to 19 nuclear genes were conducted. The resulting phylogeny shows strong support for monophyly of Yponomeutoidea sensu Kyrki (1990), when the supposed lyonetiid subfamily Cemiostomiinae is excluded. All ten yponomeutoid families as re-defined by van Nieukerken et al. (2011) were strongly supported as monophyletic, if Cemiostomiinae and Scythropia are excluded. Relationships among the families, including deeper splits within Yponomeutoidea, are also mostly well resolved. Our results provide new insights on the circumscription and classification of Yponomeutoidea, and on the evolution of their host plant associations and biogeography.

Keywords:*Lepidoptera, Yponomeutoidea, molecular phylogeny, evolution*

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A preliminary phylogenetic framework of Chalcosiinae (Lepidoptera: Zygaenoidea, Zygaenidae) and the evolution of chemical defense and mimicry

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The chalcosiine zygaenid moths constitute one of the most striking groups within the lower-ditrysian Lepidoptera, with highly diverse mimetic patterns, chemical defence systems, scent organs, copulatory mechanisms, hostplant utilization and diapause biology, plus a very disjunctive biogeographical pattern. A previous study using morphological characters and a very dense taxon sampling strategy reveals that neither the family Zygaenidae nor the subfamily Chalcosiinae is monophyletic. Although the major lineages within the Chalcosiinae sensu stricto are well-supported, the internal relationships between them, however, are still obscure. The present study shows a preliminary results of their phylogenetic analysis using 2 genes from 159 species and 2 Callizygaeninae species as the outgroup taxa. The phylogenetic pattern shows that Agalopini is the sistergroup of the remaining genera. Chalcosiini represents the most derived group with reduced male genitalia and developed A8. Cyclosiini is monophyletic but the sistergroup varies with different analyses. Arbudas is placed with Corma and thus a lineage that was not recovered by morphological analysis is revealed. The new phylogenetic hypothesis can be used to investigate the evolution of specific character sets, such as scent organs of adults, chemical defense, apodemal system of male genitalia, copulatory structures, and sexual dimorphism.

Keywords:*mimicry, polymorphism, androconia, chemical defense, cyanide*

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A molecular phylogeny for the pyraloid moths (Lepidoptera: Pyraloidea) and its implications for higher-level classification

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We present the first detailed molecular estimate of relationships across the subfamilies of Pyraloidea, and assess its concordance with previous morphology-based hypotheses. We sequenced up to five nuclear genes, totaling 6633 bp, in each of 42 pyraloids spanning both families and 18 of the 22 subfamilies, plus up to 14 additional genes, for a total of 14,826 bp, in 22 of those pyraloids plus all 24 outgroups. Maximum likelihood analyses yield trees that differ little among data sets and character treatments and are strongly supported at all levels of divergence. Subfamily relationships within Pyralidae, all very strongly supported, differ only slightly from a previous morphological analysis, and can be summarized as Galleriinae + Chrysauginae (Phycitinae (Pyralinae + Epipaschiinae)). In Crambidae the molecular phylogeny is also strongly supported, but conflicts with most previous hypotheses. Among the newly-proposed groupings is a "wet-habitat clade" comprising Acentropinae + Schoenobiinae + Midilinae, and a provisional "mustard oil clade" containing Glaphyriinae, Evergestinae and Noordinae, in which the majority of described larvae feed on Brassicales. Within this clade a previous synonymy of Dichogaminae with the Glaphyriinae is supported. Evergestinae and Noordinae are synonymized with Glaphyriinae, which appear to be paraphyletic with respect to both. Pyraustinae and Spilomelinae as sampled here are each monophyletic but form a sister group pair. Wurthiinae comprising the single genus Niphopyralis Hampson, which lives in ant nests, are closely related to, apparently subordinate within, and synonymized with, Spilomelinae.

Keywords:*Pyraloidea, Molecular phylogeny*

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S104M20

Systematics, Phylogeny and Zoogeography

Phylogenetic and comparative studies of tiger moths (Lepidoptera: Erebidae)

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Despite the important role of tiger moths in chemical ecology and behavioral studies, few molecular investigations of arctiine phylogeny exist. Preliminary molecular studies support the monophyly of Arctiini, Lithosiini, and Syntomini but do not support the limits of all subtribes within Arctiinae. To test the monophyly of tiger moth subtribes and address the evolution of tiger moth life history traits, a phylogenetic hypothesis of Arctiinae was generated based on DNA sequence data from 4 genes for 70 taxa. We examined origins of pyrrolizidine alkaloid (PA) associations and the evolution of PA pharmacophagy in adults. This study also investigated whether specialized proboscis structures are associated with adult pharmacophagy.

Keywords:*tiger moth, feeding behavior, chemical ecology, behavior*

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Repeated evolution of early and late winter geometrid moths

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Diversification during Quaternary climatic oscillation is demonstrated in various taxa. The refugial isolation hypothesis, which is geographic isolation by restricted gene flow between different refugia, has been widely supported as a mechanism of diversification during cool climatic period. This indicates dominant role of refugial isolation during climatic oscillation. Wide acceptance of refugial isolation hypothesis may dependent on easiness to detect the records of past distribution. Other speciation mechanism would also contribute to diversification in Quaternary. Our previous works demonstrated that a winter geometrid moth is diverged in cool habitat by differentiation of reproductive season whereas are not differentiated in warm habitat. It indicates that intensive climate oscillation in Quaternary causes diversification of winter geometrid moths. Here we show that a winter moth genus, Inurois, have been diversified by repeated differentiation of reproductive season and hybridized between the species with different reproductive seasons in Quaternary. This result is compatible with the hypothesis. Moreover, our result suggests that two early winter moths, which diverge from different lineages, united into single species, suggesting the important role of climatic oscillation than geographic isolation. Our results suggest that the diversification during Quaternary climatic oscillation is needed to study from various perspectives.

Keywords:*allochronic isolation, speciation, Quaternary, introgressie hybridization*

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Biogeography of the Pedaliodine complex: Are Pedaliodes complex genera consequence of horizontal or vertical events of reproductive isolation?

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The Pedaliodes species complex is one of the most diverse groups of butterflies. With over 270 species and 17 genera, the majority of these species to be found in the high Andean mountain (over 2000 m altitude). In 1986, Adams proposed a model to explain the diversification of the group, based on the altitudinal migration of different vegetation types in the Andes during the Pleistocene glaciations. However, a phylogenetic analysis based in morphology and the geographical distribution of these butterfly species suggests a closer connection to the horizontal expansion and reduction of forests and other types of vegetation areas in South America during the Pleistocene, in the sense of the Refuge Pleistocene Theory of Haffer (1969). To produce the phylogeny, we analyzed 17 genera and 45 species of the complex. The phylogenetic analysis (TNT) produced only one obtained tree (555 steps, CI= 0.23, RI= 0.53). Major conclusions were: the Pedaliodes complex is a monophyletic group, where the genus Dangond was the sister group of the other genera of the Pedaliodes complex. Out of the 17 genera covered in the present study, just Panyapedaliodes, Pherepedaliodes, Praepronophila, Redonda, and Steromapedaliodes, were natural groups. As a consequence, both, a phylogeny obtained on morphological evidence and the geographical distributions of the valid genera, are closer with the process of horizontal geographical isolation than with the vertical geographical isolation process.

Keywords:*Radiation, Speciation, Tropical High Mountain, Biodiversity.*

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S104M23

Systematics, Phylogeny and Zoogeography

Molecular systematics of the butterfly tribe preponini (Nymphalidae: Charaxinae)

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The nymphalid butterfly tribe Preponini includes some of the most spectacular Neotropical butterflies, but their taxonomy remains poorly resolved. Recent studies of Nymphalidae phylogeny have suggested that both the tribe and several genera might not be monophyletic, but to date taxon sampling has not been sufficiently comprehensive to allow informed revision of the group's systematics. We therefore conducted the first complete species-level phylogenetic study of the tribe to establish a firm higher classification. We used DNA sequence data from three genes, two mitochondrial genes, Cytochrome Oxidase subunits I and II (COI and COII), and the nuclear gene, Elongation Factor - 1 alpha (EF-1α), to reconstruct the phylogeny of the tribe using Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI). We included 48 individuals representing the 22 recognized Preponini species, and 25 outgroup taxa to explore taxonomic limits at different levels. Firstly, we found that Anaeomorpha splendida Rothschild, 1984 never formed a clade with remaining Preponini, so that maintaining monophyly of the tribe requires Anaeomorpha's exclusion and we thus reinstate the tribe Anaeomorphini [stat. rev.]. Secondly, we investigated generic limits, in particular the relationship of Noreppa Rydon, 1971 to Archaeoprepona Fruhstorfer, 1915, and Agrias Doubleday, [1845] to Prepona Boisduval, 1836. The molecular results coupled with previous morphological studies suggest that Noreppa should be synonymized with Archaeoprepona [stat. rev.] and Agrias with Prepona (syn. nov.). This study also found a number of cases of significant DNA sequence divergence and paraphyly or polyphyly within putative species that require further taxonomic attention.

Keywords:*Systematics, Preponini, Agrias, Prepona, Archaeoprepona, Noreppa, Anaeomorpha*

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S105TU01

Systematics, Phylogeny and Zoogeography

Timing the diversification of beetles (Coleoptera)

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Beetles (Coleoptera) exhibit extraordinary ecological and taxonomic diversity. However, our understanding of the evolutionary history of this diversity is in its infancy. This is in large part due to the lack of a relatively comprehensive and well-supported phylogeny for beetles. In this presentation I will present a brief overview of beetle macroevolution based on analyses of DNA sequence data conducted as part of the Beetle Tree of Life Project. Specifically, I will present evidence for the rapid radiation of beetle suborders and for the timing of origin and diversification of select beetle families and certain other higher-level taxonomic groups. I will briefly discuss the timing of beetle diversification relative to major events in earth's history, including the rise of flowering pants to ecological dominance.

Keywords:*Coleoptera, diversification*

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S105TU02

Systematics, Phylogeny and Zoogeography

Evolution of cleptoparasitic strategy in the bee family Megachilidae

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While the majority of solitary bees are nest-building, a number of lineages are recognized as cleptoparasites. These so-called "cuckoo bees" neither build nor provision their own nests but rather lay their eggs in nests prepared by a host bee. In general, the pollen stored in the nest of the host is not sufficient to sustain the development of both the host larvae and those of the cleptoparasite; in order to eliminate the competition for resources presented by the hosts' larvae, the cleptoparasite usually kills the hosts' larvae. To this end, cleptoparasitic bees have evolved two distinct strategies. In the first, the adult female cleptoparasite removes or kills the host eggs and larvae; in the second, one or more cleptoparasitic larval instars, highly aggressive and often armed with long sharp mandibles, kill the host eggs and larvae. While cleptoparasitic lineages typically display either one strategy or the other, certain lineages in the bee family Megachilidae appear to contain both modes of cleptoparasitism. We assembled a large, multi-locus dataset for Megachilidae and used this family as a model system to trace the evolution of cleptoparasitic strategy, as well as the evolutionary transition between nest-building bees and cleptoparasites. The results of maximum likelihood and Bayesian-based analyses reveal a clear pattern in the evolution of cleptoparasitic behavior; our results also indicate a correlation between the ages of cleptoparasitic clades and the strategies they employ.

Keywords:*dating analysis, cleptoparasite, solitary bees, Megachilidae*

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S105TU03

Systematics, Phylogeny and Zoogeography

Divergence time estimates for therevoid flies (Diptera: Asiloidea)

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Divergence time estimates can be influence by the inclusion of fossils in morphological data. We present the results of a phylogenetic analysis and and divergence time estimates for four families of asiloid flies representing the therevoid clade: Scenopinidae, Apsilocephalidae, Evocoidea and Therevidae. By combining morphological and molecular data we explore the effects of inclusion of different fossil taxa on phylogram topology and divergence time estimation. Fossil taxa include representative of three of the four families from Mesozoic and Cretaceous age amber deposits, including a putative stem group taxon from Burmese amber.

Keywords:*Therevidae*

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S105TU04

Systematics, Phylogeny and Zoogeography

300 millions of evolution: Divergence dating in Orthoptera (Arthropoda: Insecta)

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With more than 23,000 valid species, Orthoptera is the most diverse member of the group of polyneopteran insect orders. Despite the familiarity, the phylogenetic relationships within Orthoptera are poorly understood because of a long history of conflicting taxonomic classification schemes based on different character sets. In this presentation, I present a robust higher-level phylogeny of Orthoptera based on complete mitochondrial genomes and four nuclear genes. Using the extensive fossil evidence known from the Carboniferous to the Tertiary, I estimate divergence time for the major groups within the order. The analysis suggests that the current diversity of Orthoptera is a result of both gradual and rapid radiation followed by a number of extinctions. The current distribution is a result of both deep vicariance and recent dispersal. Therefore, the divergence dating reveals a dynamic evolutionary history of this fascinating group of insects.

Keywords:*Orthoptera, Phylogeny, Fossil*

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S106TU01

Systematics, Phylogeny and Zoogeography

Wing base morphology and phylogeny of Paraneoptera

Kazunori Yoshizawa
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The wing base structure of insects is composed of many sclerites located between the notum and wing. Very sophisticated wing movements (flapping and folding) are achieved by these sclerites, and slight modification of these sclerites can make insects flightless. Because of these constraints, this structure is known to evolve very slowly. Thus, the wing base structure should contain useful phylogenetic information for uncovering deep insect divergence. Characters selected from the wing base have been applied to estimating the higher level phylogeny of Paraneoptera. These studies have provided support for Paraneoptera + Holometabola (Yoshizawa, 2011: Syst. Ent.), monophyly of Paraneoptera, Thysanoptera + Hemiptera (= Condylognatha), Fulgoromorpha + Cicadomorpha (= Auchenorrhyncha) (Yoshizawa & Saigusa, 2001: Syst. Ent.), monophyly of Cicadomorpha, and Cicadoidea + Cercopoidea (Yoshizawa & Wagatsuma, in press: Ent. Sci.). Most of these relationships have also received support from molecular phylogenies, but monophyly of Paraneoptera has not been recovered using molecular evidence. Support values for non-monophyly of Paraneoptera from the molecular data are typically weak, whereas the wing base characters provided a total of 9 non-homoplasious autapomorphies of Paraneoptera. Therefore, monophyly of Paraneoptera can be regarded as highly plausible and deserves further examination using additional molecular data.

Keywords:*Wing base structure, phylogeny, Paraneoptera*

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S106TU02

Systematics, Phylogeny and Zoogeography

Mitochondrial genomics of Paraneoptera

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The mitochondrial genome of insects is remarkably stable through evolutionary time, with rearrangements uncommon in almost all orders. The Paraneoptera are the great exception to this stability ? major rearrangements (those involving protein-coding or rRNA genes) have been recorded in all 4 paraneopteran orders. The majority of Hemiptera species examined to date are not rearranged, however a series of synapomorphic rearrangements are found in whiteflies (Aleyrodidae). The single Thysanoptera mt genome sequenced is high rearranged. The greatest diversity of genome arrangements and structures occurs in the Psocoptera/Phthiraptera where there is an evolutionary trend towards increasing rates of genome rearrangement and the evolution of a multichromosomal mt genome. The evolution of mt genomes within the Paraneoptera and the underlying genetic causes will be discussed.

Keywords:*Genomics, Phylogenomics*

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S106TU03

Systematics, Phylogeny and Zoogeography

A timescale for the evolution of lice

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In the past decade substantial progress has been made at resolving the phylogeny of parasitic lice (Insecta: Phthiraptera). Much of this work has been driven by a desire to compare the phylogenetic histories of lice with their obligate avian and mammalian hosts. These studies have identified a number of louse taxa that show evidence of cospeciation with their hosts. Cospeciation events are important as they provide an internal time calibration on the evolution of lice. The recent discovery of two important fossils shed light on the age of lice, independent of their hosts. In this presentation I review recent work that use these cospeciation and fossil calibration points, together with data from multiple genetic markers, to establish the age of the major lineages of parasitic lice. These data demonstrates that the major families of lice began to radiate before the K?Pg boundary. The diversification of extant chewing lice parasitizing mammals postdates the K?Pg boundary, in contrast to the Cretaceous diversification of their avian infesting relatives. This suggests parasitic chewing lice were first acquired by birds and later diversified on mammals. Parasitic lice use their host's pelage or plumage as both a habitat and a food resource and host specificity can be attributed to phenotypic variation of host hair and feathers. Given the diversification times presented here and the early origin of feathers, parasitic lice probably infested feathered theropod dinosaurs.

Keywords:*louse, lice, Phthiraptera, phylogeny, dating, age*

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Phylogenomics of lice using next-generation sequencing

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New DNA sequencing methods can produce billions of base pairs of DNA sequence in a highly automated fashion for a fraction of the costs of traditional targeted sequencing. These technologies have incredible potential for providing genomic scale data sets for phylogenomic analysis. A Target Restricted Assembly Method (TRAM) was applied to Illumina sequence reads for 20 species of Psocodea using the human body louse (Pediculus humanus) as a reference. These reads were mined and assembled for genes proven to be 1:1 orthologs across insect genomes sequenced to date. A phylogenomic data set was produced and analyzed for a small sample of the 1024 potential ortholog genes. Analyses of these data indicated that mammalian Ischnocera (Trichodectidae) are sister to other mammalian lice (Rhynchophthirina and Anoplura), making Ischnocera paraphyletic. Parasitic lice were also paraphyletic, indicating multiple origins of parasitism. These analyses indicate considerable potential for next-generation sequencing technologies to be used to generate genomic scale data sets, even when material not suitable for RNA extraction is used.

Keywords:*phylogenomics, Phthiraptera, Psocodea, systematics*

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S106TU05

Systematics, Phylogeny and Zoogeography

Thripin' Awesome: Phylogeny and mitochondrial genomics of Thysanoptera

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Encompassing nearly 6,000 species, Thysanoptera has two suborders (Tubulifera and Terebrantia) with nine extant families but their phylogenetic relationships are poorly understood. Here, five genetic loci (18S, 28S, Histone 3, Cox I, and Tubulin alpha 1) for 101 species representing six of the nine extant thrips families. Trees were reconstructed using parsimony and model-based methods for the combined data set. Thrips are suspected of having a high degree of rearrangement within the mitochondrial genome, two mitochondrial genomes were sequenced using 454 pyro-sequencing for one Tubuliferan species and one Frankliniella species. The objectives of this study were to (1) test the monophyly of the suborders Tubulifera and Terebrantia; (2) elucidate relationships among the thrips families; and (3) investigate the evolution of gene rearrangements in the mitochondrial genome. Our analyses strongly support the monophyly of both suborders Tubulifera and Terebrantia. Phlaeothripidae Aeolothripidae, Melanthripidae and Thripidae are all monophyletic. The relationship between Aeolothripidae and Merothripidae to the rest of Terebrantia is equivocal. Four of the six subfamilies are recovered as monophyletic. The two largest subfamilies, Phlaeothripinae and Thripinae, are paraphyletic and require further study to understand relationships within them. Patterns of mitochondrial genome rearrangements in light of relationships are suggestive of strong phylogenetic signal in gene rearrangements.

Keywords:*Thrips, Franklinella, Tubulin-alpha, Tubulifera*

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Phylogeny and evolution of Aphidini (Sternorrhyncha)

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Aphids (Hemiptera: Aphididae) are well known as micro-insect pests, which are very specific to their host plants, and most of them have successfully maintained parthenogenetic generations cyclically or permanently. In the world, the approximately 5,000 described aphid species belong to the family Aphididae. The diversification of host plants, especially angiosperms, has played an important role in their evolution. Here, I would like to present macroevolutionary patterns of three major aphid groups, Aphidini, Macrosiphini, and Pterocommatinae, which are the most diverse in the world and constitute more than 60% of the total species. These major lineages demonstrate the evolutionary history of aphids interacting with their host plants. Major questions about aphid evolution include origins of host alternation as well as age and patterns of diversification in relation to host plants. To address these questions, we reconstructed the phylogeny using a combined dataset of one nuclear and four mitochondrial DNA regions. A molecular dating approach, calibrated with fossil records, was used to estimate divergence times of these taxa. Based on phylogenetic and dating analyses, most generic divergences in Aphidinae occurred in the Middle Tertiary when primary hosts, mainly Rosaceae, were diverging, whereas species-level divergences were related with diversification of secondary hosts such as Poaceae in the Middle to Late Tertiary. Most generic divergences in Aphidini occurred in the Middle Tertiary, and species-level divergences occurred between the Middle and Late Tertiary. My presentation will be mainly focused on the evolution of host alternation and co-diversification between aphids and their host plants.

Keywords:*Aphids, Aphididae, Aphidini, Aphis, Phylogeny, Molecular dating*

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Paraneoptera: Traits and evolution of wing venation pattern

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Monophyly of Paraneoptera is widely accepted, but two different patterns of wing venation are currently supposed to be present in each of the three major groups (Psocodea, Thripida, Hemiptera). This is unlikely compared to the situation in other insects where only one pattern exists per order. We propose for all Paraneoptera a new interpretation of wing venation pattern, assuming that the convex CuA gets fused with the common stem of median and radial veins at or very near to wing base, after separation from concave CuP, and re-emerges more distally from R+M stem. Thereafter, the vein between concave CuP and CuA is a specialized crossvein called 'cua-cup', proximally concave and distally convex. We show that despite some variations, i.e. cua-cup can vary from absent to hypertrophic; CuA can re-emerge together with M or not, or even completely disappear, this new interpretation explains all situations among all fossil and recent paraneopteran lineages. We propose that the characters 'CuA fused in a common stem with R and M' and 'presence of specialized crossvein cua-cup' are venation apomorphies that support the monophyly of the Paraneoptera. In the light of these characters we re-interpret several Palaeozoic and early Mesozoic fossils that were ascribed to Paraneoptera, and confirm the attribution of several to this superorder, as well as possible attribution of Zygopsocidae (Zygopsocus permianus Tillyard, 1935) as oldest Psocodea. We discuss the situation in extinct Hypoperlida and Miomoptera, suggesting that both orders could well be polyphyletic, with taxa related to Archaeorthoptera, Paraneoptera, or even Holometabola.

Keywords:*Psocodea, Thripida, Hemiptera, wing venation*

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S106TU08

Systematics, Phylogeny and Zoogeography

Phylogeny of Hemiptera based on the sequences of mitochondrial genomes

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Hemiptera is the largest order in Paraneoptera and the fifth largest in Insecta. Disputes about hemipteran phylogeny have long focused on the monophyly of Auchenorrhyncha or the relationships between the suborders Fulgoromorpha, Cicadomorpha, Coleorrhyncha and Heteroptera. Two mitochondrial genomes of Coleorrhyncha were provided in this study. All suborders of Hemiptera were smampled in the study. Two types of data, amino acid sequences and nucleotides of various combinations between protein coding genes, tRNAs and rRNAs of mitochondrial genomes, were used to infer the phylogeny of Hemiptera. In total 27 taxa of Paraneoptera were sampled, 24 of them being hemipterans. Three different phylogenetic methods, Bayesian inference, maximum likelihood and maximum parsimony analyses, were employed. The results question the the monophyly of Homoptera and Auchenorrhyncha. The relationship of Cicadomorpha + Heteroptera is always stable in the results with different combinations between data types and phylogenetic methods. In evaluating the relative contribution of each gene, the phylograms generated by single genes, CO1, ND1, ND2, ND4 and ND5 respectively, well matched the tree yielded from the combined datasets. In light of the taxon-sampling sensitivity of trees based on mitochondrial genomes, the results need to be tested with more data from nuclear genes.

Keywords:*Hemiptera, phylogeny*

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S106TU09

Systematics, Phylogeny and Zoogeography

Phylogeny of Heteroptera - combined morphological and molecular perspective

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With more than 40,000 described species in 86 families and 7 infraorders, Heteroptera or "true bugs" are one of the megadiverse phylogenetic lineages of animals. Their morphological and ecological diversity rivals any suprafamilial group of insects as shown by their successful occupation of most major ecosystems on Earth ? they skim the surface of the open oceans, live as temporary and permanent ectoparasites in the fur of bats, are kleptoparasitic on spider webs, and are detritivores on the tops of active volcanoes. Phylogenetic hypotheses are pivotal for understanding heteropteran diversity and essential for testing current classifications and evolutionary hypotheses. We here present the first combined morphological and molecular phylogenetic analysis of Heteroptera since the combined study by Wheeler, Schuh, and Bang (1993). This analysis aims on investigating recently proposed relationships, among them the potential sister-group relationship of Nepomorpha, the aquatic bugs, with all remaining Heteroptera. In addition, we present a combined molecular and morphological analysis that focuses on examining relationships within the Cimicomorpha, the largest heteropteran infraorder, with emphasis on the position of Pachynomidae and monophyly of Nabidae. We conclude that incorporating morphological data into large-scale phylogenies has never been more important.

Keywords:*Heteroptera, phylogeny*

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Evolution of traumatic insemination in cimicoid true bugs

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Traumatic insemination (TI), an extraordinary copulation strategy, is most prevalent in cimicoid true bugs (Insecta: Hemiptera) among invertebrates including insects. As evolutionary defensive mechanisms, not only a number of cimicoid females, but some males in response to homosexual mating attempts have evolved special morphological structures, the paragenital system (PS), which is known to reduce physical damage and increased risk of bacterial infection associated with TI. However, some cimicoids do not have developed PS despite of being engaged in TI. Here, we resolved the evolutionary history of TI and the females' adaptations by conducting molecular phylogenetic analyses and comparative studies based on the phylgenetic relationships. We found a sister group relationship of Curaliidae + Lasiochilidae, which are the only groups among Cimicoidea that are likely in non-TI. Divergence time estimates revealed that TI is a behavior of antiquity that first evolved in the Early Jurassic 188Mya (95% highest posterior density = 157-221). Furthermore, ancestral character state reconstructions indicated that TI evolved only once from standard insemination in Cimicoidea. To investigate whether TI evolution might have been related to other unique biological traits of cimicoids such as parasitism, and to test the hypothesis of correlation between TI and PS, correlation tests and contingency analyses were conducted. The results revealed that only TI is correlated with PS among the biological traits, and that evolutionary acquisition of PS was driven by selection of TI. This study also suggests convergent evolution of the females' morphology (PS) have occurred as females were adapting to TI.

Keywords:*bedbug, Cimicoidea, contingency analysis, convergent evolution, traumatic insemination, flower bugs, divergence dating, homosexuality, paragenital system, molecular phylogeny*

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Systematics, Phylogeny and Zoogeography

Vertical transmission and cospeciation of gut symbionts in heteropteran stinkbugs

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Many insects harbor mutualistic bacterial symbionts endocellularly in the specialized symbiotic organ, called bacteriome, and vertically transmit the symbionts from females to offspring. In such insect groups as aphids, tsetse flies, weevils, etc., molecular phylogenetic analyses have shown that the symbionts are monophyletic and the symbiont phylogeny mirrors the host phylogeny, suggesting a single bacterial infection in the common ancestor and subsequent host-symbiont cospeciation. The majority of plant-sucking stinkbugs (Heteroptera: Pentatomomorpha) do not possess the bacteriome but harbor bacterial symbionts extracellularly in the lumen of the midgut crypts. The symbiotic associations are generally mutualistic, wherein symbiont-deprived nymphs suffer high mortality, retarded growth, and/or reduced body size. Stinkbugs of the superfamily Pentatomoidea vertically transmit their gut symbionts by either of the post-hatch mechanisms, egg surface contamination, coprophagy, and symbiont capsule provisioning. Notably, recent studies have revealed that different pentatomoid families exhibit distinct evolutionary patterns in their host-symbiont intimacy. In the families Acanthosomatidae, Plataspidae and Urostylidae, the gut symbionts formed a monophyletic group and cospeciated with their hosts. In the families Cydnidae, Pentatomidae and Scutelleridae, by contrast, the gut symbionts are polyphyletic and exhibit no host-symbiont co-speciation, suggesting occasional symbiont acquisitions and/or replacements in their evolutionary course. Comparative studies on these stinkbugs, their gut symbionts, and interactions between them would provide novel insights into various evolutionary aspects of the mutualistic gut symbiotic associations. We argue these evolutionary patterns in relation to the anatomical traits of their symbiotic systems and the mechanisms for vertical transmission of their gut symbionts. [Supported by BRAIN]

Keywords:*symbiosis, Pentatomoidea, midgut, bacteria*

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The Flytree of life: Resolution and radiation of higher- level fly phylogeny

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FLYTREE is a consortium of world dipterists engaged in a collaborative research project initiated by the US National Science Foundation Assembling the Tree of Life (AToL) program. During its six-year funding term, this project combined the efforts of over 19 laboratories in 6 countries. Data collection and phylogenetic analyses were divided in tiers: Tier 1 includes 45 species across the order and several outgroups sampled for approximately 30kb of aligned genetic data from 14 nuclear genes, full mitochondrial genomes and 371 scored morphological features; Tier 2 includes 202 taxa comprising at least one species from nearly all families, sampled for 5 nuclear genes. Combined molecular data from the 1st and 2nd Tiers form the largest matrix yet completed for Diptera. Tier 3 studies involve detailed phylogenetic analysis within dipteran clades achieved through a network of interacting research programs. These studies enabled 30+ published phylogenetic studies that treat over 1000 fly taxa for molecular and/or morphological data. Here, major findings of the FLYTREE project are presented, highlighting specific hypotheses about the origin, age, and diversification of fly lineages and emphasizing modern approaches in large dataset construction and analysis. Remaining questions serve to direct, guide, and focus the search for new evidence from genomic data harvests and morphological surveys. The FLYTREE project continues through the shared research activities of the Diptera systematics community and provides a successful model for research collaboration toward resolution of complex and hyperdiverse areas of the tree of life.

Keywords:*Diptera, Phylogeny, molecular systematics*

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The Flytree morphology: A new phylogenetic estimate from exemplar-based analysis across the order

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The Diptera are one of the largest insect Orders, with over 150,000 described species in over 10,000 genera classified in 150 recognised families. FLYTREE is a large international consortium funded by the US National Science Foundation's Assembling the Tree of Life initiative to estimate Dipteran phylogeny. FLYTREE has assembled the largest supermatrices of Diptera, including both molecular and morphological components. 371 morphological characters scored for 42 exemplar fly species spanning the order, and mecopteran outgroups. Trees based on non-additive parsimony analysis of the morphological supermatrix largely confirm Hennig and other author's previous findings, and are consistent with mixed-model Bayesian analysis of concatenated molecular sequences. Well-supported clades (Bremer Support indices > 10) include Diptera, Brachycera, Cyclorrhapha, Schizophora, Calyptrata and Oestroidea. The Nematocera, Orthorrhapha, Aschiza and Acalyptrata are found to be paraphyletic, and support for relationships in these regions of the tree is also poor. The results suggest rapid bursts of fly diversification at the end of the Permian, in the Jurassic, and at the Cretaceous/Tertiary boundary.

Keywords:*Diptera, morphology, phylogeny, relationships, evolution*

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Reciprocal illumination between morphology and molecules in basal Diptera phylogeny

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Morphology and molecules quite frequently result in conflicting topologies?as also different partitions of morphological or molecular data. This is good. Incongruence is useful in terms of compelling reexamination of prior assumptions. The molecular phylogeny of Diptera published by Wiegmann and collaborators in 2011 conflicts in some extent with older molecules philogenies?e.g., about the position of the Tipulomorpha, the Scatopsoidea and other groups. Some of the new molecular hypotheses are largely congruent with information, e.g., from thorax morphology, but there are still important conflicts. There is strong evidence that a single event of detachment of the meron from the midcoxa occurred?a major change in the evolution of thorax morphology in Diptera?, a feature plesiomorphic in Tipulomorpha (including Trichoceridae), Bibionomorha (including Anisopodoidea and excluding Scatopsoidea), and Culicomorpha, but derived in Brachycera, Psychodomorpha and Blephariceromorpha. On the other hand, the exclusion of "Manotidae" from the Mycetophilidae based on molecular data was surprising at a first sight. Reexamination of the wing venation of the plesiomorphic genus Paramanota shows a long, complete cubital fork, a feature derived in almost all mycetophilids?showing that in this case morphology may have been wrong in the past. Clades of the basal Diptera in the new molecular phylogeny are analyzed about their support in morphological evidence. A closer contact between morphology and molecule data shortens the path to more robust phylogenies. This does not mean only total evidence, but especially reciprocal illumination for double checking ad hoc hypotheses of homology in morphology and molecular data.

Keywords:*Diptera, phylogeny, reciprocal illumination, ad hoc hypotheses, homology*

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Molecular phylogeny of dark-winged fungus gnats (Diptera: Sciaroidea: Sciaridae) and the evolution of larval feeding habits

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We present the first molecular phylogeny of the Sciaridae. This phylogeny is based on maximum likelihood, maximum parsimony, and Bayesian analyses of 4,809 base pairs (bp) of DNA sequences from two mitochondrial (COI and 16S) and two nuclear (18S and 28S) genes for 100 taxa including the outgroup. According to the present phylogenetic analyses, Sciaridae involve three subfamilies and two genus groups: Sciarinae, Cratyninae, Megalosphyinae, Lycoriella group, and Chaetosciara group. Molecular results are largely congruent with former hypotheses based on morphological data with respect to the monophyly of genera and subfamilies (Sciarinae, Megalosphyinae, and part of "Lycoriellinae") sensu Menzel and Mohrig (2000). However, the subfamily Cratyninae sensu Menzel and Mohrig (2000) could be paraphyletic and the Chaetosciara group is separated from the subfamily Sciarinae sensu Menzel and Mohrig (2000). Bradysia, Corynoptera, Leptosciarella, Lycoriella, and Phytosciara could be paraphyletic groups. Furthermore, the ancestral larval feeding state reconstruction based on Bayesian inference was saproxylic (detritophagous + xylophagous) for the Sciaridae. In contrast, the larval feeding habits of the common ancestors of Phytosciara and Bradysia were inferred to be phytophagous. Therefore, shifts in larval feeding habits from saproxylic to phytophagy occurred within the Sciaridae at least twice.

Keywords:*Sciaridae, molecular phylogeny, decomposer fly, sciarid larval feeding habits, ancestral states*

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Phylogeny and biodiversity of therevoid flies (Diptera: Asiloidea)

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Four families of asiloid flies comprise the therevoid clade: Scenopinidae, Apsilocephalidae, Evocoidea and Therevidae. Previous investigations into understanding the evolutionary history of this group have been piece-meal and focused on adult morphology, molecular sequence data and even include a super-tree analysis. Despite this, our knowledge of the group is still fragmentary. A summary of our knowledge of therevoid clade phylogeny is presented, including results of recent large-scale analyses of combined molecular and morphological data, including estimates of divergence times including fossil taxa. Relationships among the families and subfamilies of Scenopinidae and Therevidae are examined using statistical measures. Therevidae represent the bulk of species level diversity in the clade, with numerous new species being described presently, especially from the Australasian region. Results of cybertaxonomic studies attempting to describe this species rich fauna expediciously are presented.

Keywords:*Therevidae, Scenopinidae, phylogeny*

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Higher flies fly high - mitogenomics of Schizophora through next generation sequencing

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The order Diptera represent 12‰ of the insects biodiversity described, including species adapted to different environments, acting as pathogen vectors, parasites, decomposers and pollinators, in addition to model organisms. Despite exponential increase of sequencing datasets, arthropods have been omitted from recent genomic studies. With the availability of the new small-scale genome sequencing instruments from Ion Torrent (Personal Genome Machine) and Illumina (MiSeq) we generated the complete mitochondrial DNA (mtDNA) from long-PCR products of 24 fresh and preserved specimens of Schizophora clade (Diptera: Brachycera) under the same pipeline for data production and analysis. The assemblings were generated with >300 thousand reads for each species, producing a coverage of >2500X of the mtDNA. The mitochondrial genome is the most used molecular marker in evolutionary studies in metazoans, but the access to the whole sequence in different species under the same pipeline is not widespread, reflecting the diversity and difficulties in the DNA attainment of small samples and preserved specimens. The use of this approach in a broad range of species can lead the mitogenomics of invertebrates to a new standing, providing a high number of characters to be applied in evolutionary studies, molecular identification, specific primer design and increasing the assessment of the genetic diversity in different taxonomic levels. The application of these technologies enables to rapidly move from comparative genomic studies to population genomics of this important order, which plays a significant role in human health and the environment.

Keywords:*mtDNA, long-PCR, next-generation sequencing, Diptera, mitogenomics*

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Systematics, Phylogeny and Zoogeography

Outgroup relationships, basal phylogeny and classification of the family Tephritidae

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The fly family Tephritidae (Diptera) contains some of the most significant agricultural pests in the world and has some importance as a source taxon for the biological control of weeds. A well-defined phylogeny of the major lineages within this important family is presently lacking and is essential for further research on these flies. At present, six subfamilies are recognized, each containing a number of different tribes. However, characters defining these taxa are often weak, the relationships among the taxa are undefined, and classification schemes do not serve as a predictor of evolutionary relationships. Our molecular data already have significantly contributed toward the sound classification of the family Tephritidae. Some of the examples of such contributions are the confirmations of the following systematic hypothesis based on the sequences of 16S rRNA, 12S rDNA, COI and COII genes: (1) two monophyletic groups within the superfamily Tephritoidea were clearly recognized they are compatible with Willi Hennig's Pallopteroidea and Otitoidea that are not used in the contemporary higher classification; (2) a sister group relationship between Ortalotrypeta and Tachinisca, and their basal phylogenetic position within Tephritidae (3) a sister group relationship between the tribe Acanthonevrini and Phytalmiini; (4) monophyly of Taomyia plus an undescribed new genus, and their sister group relationship with the subfamily Tephritinae; (5) a possible sister group relationship of Cephalophysa and Adramini; and (6) reconfirmation of monophyletic Trypetini, Carpomyini, Tephritinae, and Dacinae.

Keywords:*Tepritoidea, Tephritidae, Diptera, Classification, mitochondria*

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The trouble with rapidly diversifying Neotropical lineages: Can the current Pipunculidae classification be incorporated into new phylogenetic hypotheses?

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Pipunculidae are important parasitoids of leafhoppers and their relatives (Auchenorrhyncha). One bizarre, autapomorphic genus (Nephrocerus) was recently discovered to attack adult crane flies (Tipulidae). This deviation from the rest of the genus is also mirrored in unique morphology and has posed a considerable challenge for phylogenetic placement. We are now testing this and other previous hypotheses using 7528 base pairs of molecular data from the mitochondrial genes COI, 12s and cyt B and the nuclear genes AATS and CAD. In addition to the placement of enigmatic taxa like Nephrocerus, questions about the utility of some characters (particularly wing vein characters) have never been fully tested. To this end, we have sampled 159 ingroup taxa to more fully test the monophyly of and relationships between existing genera. The status of several sister taxa such as Jassidophaga/Verrallia, Microcephalops/Collinias,Cephalops/Cephalosphaera and Pipunculus/Parapipunculus are explored and the status of existing subgenera are also examined and re-interpreted. The biggest challenge within the Pipunculidae has been untangling the taxonomy of the huge tribe Eudorylini. Several genera of eudorlyines have been proposed with some expectation that the original genus Eudorylas is now a paraphyletic assemblage of leftover species. A morphologicaly analysis of this group was attempted recently but this is the first time that extensive sampling using multiple independent datasets have been brought to the question. Most genera have survived this test of their monophyly but some will clearly need to be redefined and at least two new genera will be proposed.

Keywords:*Pipunculidae, phylogeny, molecular phylogenetics*

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S107TU09

Systematics, Phylogeny and Zoogeography

Explosive radiation of parasitoid flies: Phylogeny and evolution of tachinidae

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The family Tachinidae is one of the most diverse families of Diptera and it is the most important group of insect parasitoids outside the Hymenoptera. All tachinids are endoparasitoids, attacking as a group at least 14 arthropod orders. They parasitize this diversity of hosts with a remarkable array of oviposition and reproductive strategies. Despite their diversity and importance, Tachinidae have been the subject of very little phylogenetic study. Composition of, and relationships among tribes and subfamilies are obscure and the family is among the most taxonomically difficult of Diptera. Here we report on some of the initial progress of our efforts to understand the phylogenetic relationships and evolution of this remarkable family using molecular and morphological data: 1. Large scale phylogenetic analyses indicate that the Tachinidae is one of the youngest and most rapidly diversifying families of Diptera. 2. Preliminary molecular phylogenetic analysis suggests that tachinids may have arisen from within a polyphyletic Calliphoridae. 3. Both morphological and molecular data support general groupings of subfamilies Dexiinae + Phasiinae and Tachininae + Exoristinae. 4. Of these subfamilies, only Exoristinae is well supported as monophyletic in both molecular and morphological analyses. 5. The evolution of egg type (ovipary, ovolarvipary) appears to be highly labile, and morphological data suggest that early hosts of tachinids were hemimetabolous with subsequent explosive diversification onto larval holometabolous hosts.

Keywords:*parasitoid, adaptive radiation, molecular phylogenetics, morphological phylogenetics, Diptera, Tachinidae*

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S107TU10

Systematics, Phylogeny and Zoogeography

Mining Genbank to resolve Orthorrhapha: A supermatrix analysis of lower Brachycera

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Fly evolution has undergone at least three rapid bursts of diversification, one occurring within the Lower Brachycera approximately 180 Ma. This region of the fly tree includes 23 families with a diversity of life histories: large flower visiting flies, blood feeders (Tabanidae, Athericidae, Rhagionidae), parasitoids (Bombyliidae, Acroceridae, Nemestrinidae) and predators (Asilidae). Though lower brachyceran families have traditionally been grouped together in Orthorrhapha, this group has widely been considered a paraphyly- lacking both morphological and molecular support. Recent large-scale molecular analyses of high-level fly phylogeny, however, found a monophyletic Orthorrhapha (excluding Empidoidea) to be well-supported. To test this unexpected result of orthorrhaphan monophyly, we have compiled a supermatrix of existing molecular markers from Genbank for a taxon dense sampling of lower brachycerans. This data set, including hundreds of taxa and up to 15 genes, is the largest compiled to date for the resolution of fly phylogeny.

Keywords:*phylogeny*

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S108W01

Systematics, Phylogeny and Zoogeography

Phylogenomic distribution and functions of DNA methylation in Insect genomes

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Georgia Institute of Technology, U.S.A.

DNA methylation is an important epigenetic mechanism involved in regulatory and developmental processes. Recent genome projects have revealed that functional DNA methylation systems are widespread among invertebrates, in particular, insects. This observation raises several intriguing questions. Why are DNA methylation systems absent in some insect species, such as the model insect fruitfly? What does DNA methylation do in different species? What are the evolutionary implications of DNA methylation? Comparative analyses of DNA methylation have begun to address these questions. In this talk, I will first summarize results showing the variation in DNA methylation across insects (and more generally, invertebrates), and the multi-faceted functional role of DNA methylation. In addition, I will present results indicating significant role of DNA methylation in lineage-specific adaptive evolution. Together, these studies highlight the significance of DNA methylation in insect genome evolution. Moreover, insects are emerging as excellent model systems to investigate the functional roles and evolution of DNA methylation.

Keywords:*DNA methylation, genome evolution*

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S108W02

Systematics, Phylogeny and Zoogeography

Phylogenomics and the evolution of insects - The 1KITE project

Bernhard Misof
on behalf of the 1KITE consortium, Germany

1KITE (1K Insect Transcriptome Evolution) is an international initiative that aims to study the transcriptomes of 1,000 insect species encompassing all recognized insect orders (www.1kite.org). We will address the phylogeny of insects by analyzing an unprecedented amount of nucleotide sequence data, allowing us to reconstruct the major character transformations and timing of diversifications in their evolution.. Scientists involved include experts in molecular biology, insect morphology, taxonomy, paleontology, embryology, bioinformatics, and scientific computing from eight nations (Australia, Austria, China, Germany, Japan, Mexico, New Zealand, and the US). 1KITE is divided into several subprojects focusing on specific phylogenetic groups, for example, apterygote hexapods, Odonata, Polyneoptera, Hymenoptera, Trichoptera, and Antliophora. Additionally, 1KITE includes the development of new software for data quality assessment, phylogenetic reconstruction, and molecular dating. Sequencing will be completed by the end of 2012. Preliminary analyses of the already available sequences show that the obtained data are of yet unparalleled size and quality. We were able to identify ~ 1000 1:1 orthologous genes for every transcriptome we sequenced and first results show that inferred trees have the potential to deliver a plausible backbone tree of insects with robust statistical support. The results can be expected to have an extraordinary and long-standing high impact on entomological and phylogenetic research.

Keywords:*Phylogenomics, transcriptomes, NGS*

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S108W03

Systematics, Phylogeny and Zoogeography

Phylogenomic insights into the Cambrian explosion, the colonization of land and the evolution of flight in Arthropoda

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The timing of the origin of arthropods in relation to the Cambrian explosion is still controversial, as are the timing of other arthropod macroevolutionary events such as the colonization of land and the evolution of flight. Here we assess the power of a phylogenomic approach to shed light on these major events in the evolutionary history of life on Earth. Analyzing a large phylogenomic dataset (122 taxa, 62 genes) with a Bayesian relaxed molecular clock, we simultaneously reconstructed the phylogenetic relationships and the absolute times of divergences among the arthropods. Simulations were used to test whether our analysis could distinguish between alternative Cambrian explosion scenarios with increasing levels of autocorrelated rate variation. Our analyses support previous phylogenomic hypotheses and simulations indicate a Precambrian origin of the arthropods. Our results provide insights into the three independent colonizations of land by arthropods and suggest that evolution of insect wings happened much earlier than the fossil record indicates, with flight evolving during a period of increasing oxygen levels and impressively large forests. These and other findings provide a foundation for macroevolutionary and comparative genomic study of Arthropoda.

Keywords:*Arthropoda*

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Whole genomes help resolve the subordinal phylogeny of beetles

Duane D. McKenna
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Beetles (order Coleoptera) comprise the largest order of animals. They are part of the food web in nearly every non-marine habitat and play numerous and important ecological roles. While molecular phylogenetic data have helped resolve some beetle relationships, others, particularly the interrelationships of the four beetle suborders (Adephaga, Archostemata, Myxophaga, and Polyphaga), remain uncertain. In this talk I will present results from my ongoing studies of beetle genomes, including what these data can tell us about interrelationships among the four suborders of beetles and relationships between beetles and their close relatives in other orders.

Keywords:*Coleoptera, phylogenomics, suborders*

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S108W05

Systematics, Phylogeny and Zoogeography

Resolving rapid radiations within Diptera: A phylogenomic approach

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Recent efforts to recover the fly tree of life have greatly advanced our knowledge of fly phylogeny. Fly evolution has taken place in episodic bursts, including 3 ancient rapid radiations corresponding to: lower Diptera (mosquitoes; 220 Ma), lower Brachycera (horse flies;180 Ma), and Schizophora (Drosophila, house flies; 65 Ma). Ancient lineages that underwent rapid radiations, such as these, are often left unresolved by standard phylogenetic methods. Fly model organisms used across scientific disciplines all occur within these lineages and a fully resolved phylogenetic framework is needed for contextualizing the comparative biology of flies. As systematics transitions to the regular use of large-scale gene harvests, flies are an excellent phylogenomic study system because of the wealth of existing genomic data that has already been generated as a result of their role as important model organisms, disease vectors and agricultural pests. Now we aim to explore the utility of transcriptome data to further resolve these ancient rapid radiations in fly evolutionary history. Using new and existing data, we have identified thousands of orthologs shared across deep fly divergences, from Anopheles gambiae to Drosophila melanogaster. In comparison to targeted PCR, next-gen sequencing has more rapidly and affordably provided a wealth of genes that exhibit phylogenetic utility across flies.

Keywords:*transcriptome, orthology, Diptera, phylogenomic, model organisms, comparative genomics*

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S109W01

Systematics, Phylogeny and Zoogeography

The phasmatodean tree of life: Progress and enigmas

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The phasmatodeans or stick and leaf insects comprise more than 3000 described herbivorous arthropods distributed mainly in tropical and subtropical regions. Considered to be a mesodiverse insect order, the group has thus far received relatively little phylogenetic attention. According to recent morphological and molecular studies, many of the currently recognized suborders, families, subfamilies and tribes are not monophyletic, with few exceptions. For instance, the Necrosciinae, the most species-rich traditional subfamily of stick insects, appears to be broadly monophyletic, with only a few species 'misplaced'. In contrast, the tree lobsters or Eurycanthinae, which comprise a small number of specialized ground-dwellers on Pacific islands, are polyphyletic, exhibiting a high degree of convergent evolution. In general, molecular analyses have demonstrated that phylogeny correlates better with geographic distribution than with morphological similarities. Since stick insects possess only limited dispersal capabilities, they are ideal model organisms for biogeographic research. Numerous previously unexpected lineages of stick insects have been recovered now. However, the relationships among these clades are contentious. For instance, the leaf insects (Phylliinae) frequently change their supposed phylogenetic position among Phasmatodea across different data matrices and methodologies. This problem might reflect a rapid ancient radiation of stick insects that gave rise to the diversity of extant forms and highlights the need to explore further sources of evidence.

Keywords:*Phasmatodea, stick insects*

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Paleobiology of fossil stick- and leaf-insects

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Among insects, various defensive strategies have been developed to protect them against predators. Defensive behaviours can be divided in primary and secondary defenses. Primary defenses operate regardless of the presence of a predator and thus decrease the chance of encounter with an predator, secondary defenses operate when a predator is present and increase the chances of survival of the prey. The main primary defense mechanism is crypsis. The evolution of the elaborate morphological adaptations of phasmids to the mimicking of plants is largely still unclear, but some fossils allow further insights. We give an overview of fossils which allow conclusions on the course of evolution of plant mimicking. The preserved morphological structures also allow inferences on the former behaviour of the phasmids. For example, Eocene fossils from Baltic amber (Russia) and from the fossil site Grube Messel (Germany) show basally curved fore femora. These curved femora enclose the head in the typically resting position of stick and leaf mimics, which is adopted during catalepsy or adaptive stillness at daylight. The Eocene Eophyllium messelense indicated that leaf insects descended from stick insect ancestry and illuminates the evolution of leaf mimicry. As a secondary defense mechanism, numerous extant stick insects have an autotomy mechanism that severs the leg at the femoral-trochanter joint. An Eocene stick insect from Grube Messel indicates that this behaviour had already evolved in the Eocene. Appendotomy and the capability of regenerating legs might have even influenced multiple losses of wings.

Keywords:*Phasmatodea, fossil*

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S109W03

Systematics, Phylogeny and Zoogeography

Homologies in stick-insect wing venation and taxonomic implications

Olivier Bethoux
France

Conjectures of topographic homologies in stick-insects wing venation pattern have been seldom investigated, and results are contradictory. The position of the group within insect phylogeny is a matter of debate, although nested as sister-group of orthopterans (grasshoppers, crickets and their kin) has been historically favoured. Concurrently, identification of Mesozoic (Palaeozoic?) relatives is not evident. In order to address these issues a collection of extant stick-insect wings was prepared and a comparative analysis of wing venation pattern was undertaken. Successive vein fusions are documented based on transformation series across species, and intra-specific and intra-individual variability. Cases of vein loss and vein translocation are also documented. It is argued that stick-insect hind wings have a wing venation pattern diagnostic of orthopterans, in particular a branched CuP. Putative fossil representatives are reconsidered, and closer relationships with Caelifera are hypothesized.

Keywords:*Phasmatodea, Phasmida, Orthoptera, fossil record, Conjecture of topographic homology*

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S109W04

Systematics, Phylogeny and Zoogeography

Flight in stick insects: Biomechanics and implications for ecology and evolution

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Stick insects exhibit a wide spectrum of wing size variation, which potentially implies differences in function, ecology and evolutionary history. Here we investigate the functionality of flight in stick insects using integrative approaches. First, quantitative measurements of the wing venation and membrane morphology in wings of different sizes showed a continuous structural reduction and simplification along the size gradient. Second, assisted with high-speed filming and biomechanical analysis, we revealed diverse flight performances associated with different wings, from full flapping capacity to gliding or parachuting with reduced wings. Species with better developed wings generally show greater flight capabilities, and short-wing species show distinctive features in wing flapping kinematics and aerodynamics. Comparison among different flight modes suggests a transition in the functionality of flight along with the transition in wing size. The variation of flight capability in stick insects may be ecologically related with environmental gradients. Understanding the adaptive significance of various wing designs in stick insects may help to explain the evolutionary transitions between flightless and volant forms.

Keywords:*biomechanics, glide, wing*

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S109W05

Systematics, Phylogeny and Zoogeography

Evolution and systematic position of Phasmatodea - A morphological approach

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Phasmatodea (walking leaves, stick and leaf insects) comprise around 3000 species, most of them occurring in the tropical regions. Along with Blattodea, Orthoptera, Dermaptera and some other groups they belong to the lower neopteran insects (Polyneoptera), which diversified in a very short time span in the late Carboniferous some 300 million years ago. This rapid radiation is reflected by the still unresolved relationships between the modern lower neopteran orders. Phasmatodeans have long been considered as closest relatives of Orthoptera (grasshoppers, crickets and katydids) or even as a subordinate group. However, several recent morphological studies and also analyses of molecular data indicate a sistergroup relationship between Phasmatodea and the cryptic Embioptera (webspinners). Potential synapomorphies include eggs with an operculum and an internal micropylar plate and several derived muscular features of the head.

Keywords:*Morphology, Phylogeny*

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New insights into the evolution of sperm structure in stick and leaf insects

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The sperm structure of representatives of Phasmatodea was studied by light microscopy, and scanning and transmission electron microscopy. In Timema, a putative basal phasmatodean lineage, spermatozoa are short flagellate cells about 55-60 µm in length. They are characterized by a three-layered acrosomal complex. The distinctive bell-shaped morphology of the acrosome vesicle is likely an autapomorphic trait of Timema. The flagellum has a 9 + 9 + 2 axonemal pattern, two accessory bodies, two membranous sacs, and two elongated mitochondrial derivatives. Results support the hypothesis of a monophyletic Phasmatodea, comprising Timema + Euphasmatodea. The presence of 17 protofilaments in the wall of accessory microtubules, and the laminar configuration of the flagellum are potential apomorphic groundplan features of the order. Within Phasmatodea, a key evolutionary divergence was from the conventional insect spermiogenesis and sperm structure of Timema, to the unusual spermiogenetic process and peculiar sperm structure of Euphasmatodea. As a consequence, Timema retains more sperm chatacter states found in the polyneopteran ground pattern, and is mostly characterized by plesiomorphic traits. Conversely, Euphasmatodea have evolved several derived sperm features. Putative autapomorphies of Euphasmatodea include the loss of mitochondria and membranous sacs, and the presence of two strongly expanded accessory bodies. Within Euphasmatodea, spermatozoa display major differences in cell-size, morphology of accessory bodies, and structure of the acrosomal complex.

Keywords:*Phasmatodea, Timema, Euphasmatodea, Sperm ultrastructure, Insect phylogeny*

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S109W07

Systematics, Phylogeny and Zoogeography

Affinity of phasmatodea: Embryological evidence

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Embryogenesis of Phasmatodea is characterized by mesoderm segregation of fault type, development of short germ band type, and embryo's localization on egg surface. The former two are the groundplan of Polyneoptera, and the third one, which is shared by some groups of Polyneoptera, cannot develop a plausible phylogenetic discussion. Hereat, egg features deserve a special mention. Phasmatodean eggs are characterized by an operculum at anterior egg pole, a micropyle at ventral egg side, and a polar mound at posterior egg pole. These features are unique, but only Embioptera bear a set of these features. They may be regarded as synapomorphies of Phasmatodea and Embioptera, and Eukinolabia is supported. Although the micropyle of Eukinolabia has been understood to be single, Timematodea were recently revealed to have a pair of micropyles and at least some phasmatodean forms to possess the micropyles adjacently paired. The embiopteran egg has a single micropylar tube, but its canal is flattened, often showing dumbbell-shape in cross section, as if paired micropyles were fused into one. Thus, the micropyles of Eukinolabia may be asserted to be paired as groundplan. Paired micropyles are peculiar in Polyneoptera but only found in Zoraptera. The affinity between Eukinolabia and Zoraptera is suggested. Hereat, recent information from spermatozoa may be interesting (Dallai et al., 2011): Phasmatodea and Zoraptera share the number of protofilaments of accessory tubules "17" which is singular among Polyneoptera; Eukinolabia and Zoraptera share a peculiar feature concerning the centriole adjunct. Furthermore, Embioptera and Zoraptera share an extraordinarily elongated egg tooth.

Keywords:*Phasmatodea, embryology, egg features, Embioptera, Zoraptera*

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S109W08

Systematics, Phylogeny and Zoogeography

Molecular phylogeny of stick insects based on mitochondrial genome

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Stick and leaf insects are famous for the extreme adaptation of crypsis, which can lead to morphological convergence making it difficult to infer phylogenetic relationships. In order to circumvent the morphological constraints, we present molecular phylogeny of Phasmatodea depending on mitochondrial DNA (mtDNA).There are advantages of mtDNAs for phylogenetic analyses, such as the absence of recombination, introns, and paralogs. PCR-based cloning of mtDNA is also helpful to obtain relatively large data sets. We have sequenced the mtDNAs of more than 20 phasmatodean species. All of the mtDNAs share the same gene arrangement as putative ancestral Pancrustacea mtDNA. The phylogenetic relationships inferred by the mtDNA sequences resulted in disagreement with conventional taxonomy based on morphology. For example, the molecular phylogeny did not support the monophyly of Areolatae or Anareolatae, two major groups of Phasmatodea. There are discrepancies also at the subfamily level; for example, Neohirasea, a member of Lonchodinae, was grouped with species of Necrosciinae in our analyses. The position of Timema is controversial. It has long been regarded as a basal taxon of stick insects, included in Areolatae. Our analyses showed that Timema is distantly related to the other phasmatodean species, suggesting that it will be necessary to revise the phylogenetic position of Phasmatodea in Polyneoptera.

Keywords:*mitogenome*

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Phylogeny and genome evolution in the New Zealand stick insects (Phasmatodea)

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The New Zealand stick insect fauna contains 10 genera and 23 described species, in addition to several undescribed species. We have reconstructed a phylogeny from multiple nuclear and mitochondrial genes to determine the biogeographic origins and diversification of the New Zealand stick insects. Two independent lineages were recovered both with sister taxa in New Caledonia and both of similar age. One of these lineages is monotypic and the other contains the remaining 22 species. Phylogenetic relationships among the individual gene trees vary and we discuss the significance of this incongruence and resolution of a species phylogeny. The New Zealand stick insects descended from subtropical and tropical ancestors yet now inhabit temperate environments. Two genera have species that are alpine obligates or found in lowland and alpine habitats. We have also performed analysis of transcriptome data to reveal genes that are involved temperature stress response and under selection. These latter genes are candidates for involvement in adaptation to these new environments and include genes involved in energy metabolism.

Keywords:*Stick insect, Phasmatodea, phylogeny, biogeography*

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How clasper morphology relates to genetic and behavioural isolation in the New Zealand stick insect genus *Clitarchus*

Shelley Susan Myers[1], Alice Dennis[2], Gregory I. Holwell[3], Thomas R. Buckley[4]

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A hybrid zone is the geographic area where two species meet and form offspring. It is common for the offspring of hybridising species to have reduced fitness. The theory of reinforcement predicts that prezygotic barriers will evolve between hybridising groups and there will be selection against hybridisation. In the Far North of New Zealand, the widely-distributed stick insect species C. hookeri is replaced by an ecologically similar and undescribed species of Clitarchus. These species are differentiated on the basis of male terminalia (specifically the claspers), egg morphology and mitochondrial DNA. Populations sampled between the two species show intermediate morphology. The aim of this research project is to describe the level of isolation between species of Clitarchus using interdisplinary techniques including behavioural studies, genetics and morphology. Genetic divergence and gene flow is being measured using SNP data obtained from 454 genomic reduction sequencing. Three dimensional morphometrics accurately describes differences in intra and inter-specific clasper shape. These quantitative approaches are being combined with mating experiments to provide a basis for addressing the following questions: does variation in morphology correspond to differences in mating behaviour and does morphological variation correlate with reproductive isolation?

Keywords:*Hybridisation, SNPs, Three dimensional morphometrics, Behaviour*

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The stick insects of Madagascar: Ancient lineages or recent adaptive radiation?

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Madagascar is one of our planet's leading biodiversity hotspots exhibiting an extraordinarily distinct and diverse biota. Many elements of its fauna are still poorly documented and remain largely unexplored. One example is the Malagasy stick insects (Phasmatodea) whose evolutionary history is entirely obscure. Stick insects are large terrestrial arthropods with limited direct-dispersal abilities, forming at least four distinct taxonomic groups on Madagascar, i.e., endemic families and subfamilies. Their phylogenetic affinities to continental lineages are completely unresolved. We present informative sets of molecular and morphometric data to investigate the origin and evolution of Malagasy stick insect lineages. Analysing a representative taxon sampling we demonstrate that the stick insects of Madagascar are more closely related to each other and much younger than previously suggested in traditional classifications, representing a classical example of adaptive radiation in geographic isolation. The application of a comparative morphospace analysis to the obtained phylogeny additionally provides an opportunity to compare unrelated and geographically distant radiations among phasmatodeans, for instance those of Madagascar compared to a diverse lineage of Australasian stick insects (Lanceocercata). Thereby, we detect an impressive degree of convergence between these otherwise distinct communities.

Keywords:*Phasmatodea, insects, morphospace, phylogeography, Madagascar*

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S110TH01

Systematics, Phylogeny and Zoogeography

Bio[phylo]geo[eco]graphy: The multidisciplinary nature of plant and animal geography

Malte Ebach
University of New South Wales, Australia

21st century biogeography is in a state of denial. Biologists believe that biogeography is a single scientific field with a single goal and common history - a field that can somehow be unified methodologically. Ecologists, taxonomists, molecular systematists, bacteriologists, astrobiologists, geographers, conservation biologists and palaeontologists all do "biogeography" of one kind or another, but each ask very different and often conflicting questions. To an outsider, it is hardly surprising to discover that biogeography has multiple origins that ask separate questions and do different things. Then why do some biogeographers believe that a highly diverse field can be unified, especially by a single methodology? The short answer is a lack of integration and interdisciplinary projects, virtually no exposure to biogeographical theory and history. Most important is the belief that a single term, "biogeography", originally undefined, is enough to encapsulate various disciplines, aims and practices as a single field. Rather than talk of unification, biogeographers should look to their strengths and embrace a multidisciplinary field that has a enormous potential. This talks hopes to untangle these beliefs and reveal the exciting problems that the multidisciplinary field of biogeography has still to answer.

Keywords:*Biogeography, History, Theory*

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Biogeographical units: Kinds of boundaries or individual wholes?

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The role played by areas of endemism in the study of biogeography is analogous to that of species in the context taxonomy: areas of endemism (AE) can be viewed as the fundamental units of biogeographical research. Despite various criticisms made regarding the usage of AE, delimitation of these units has proved to at least have some empirical utility. Because of that, we can think of AE as an imperfect concept, which can partly accommodate our knowledge and predictions, but that often fails to express our hypotheses about spatial evolution. Here, we promote the notion that the vicariant events are the real focus and the genuine units of biogeography, in agreement with Hovenkamp's proposals. Shifting from an AE-based approach to this alternative strategy requires attention to be directed to the boundary (or part of the boundary), instead of the area itself. These boundaries, namely 'hypotheses of vicariant boundary' (VB), can be construed as fuzzy elements of the world conveying historical information due to vicariance, best thought of as natural kinds concepts. No necessary or sufficient properties need to be ascribed to these kinds, because unique histories may have molded these units differently over time, generating distinct homeostatic property clusters in each situation. The nomological commonness among all cases of VB's, i.e., generational vicariant processes, makes these kinds relevant for the study of historical biogeography as the fundamental units of this field of investigation. The importance of VB-based biogeography will be exemplified with examples from previously conducted biogeographical research with insects.

Keywords:*areas of endemism, biogeography, vicariance, natural kinds individuals, homeostatic property cluster*

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Many oriental regions

Dalton de Souza Amorim
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Biogeography uses areas as units to produce hierarchical systems. The nature of space as biogeographical units is intrinsically different from terminals in other systems, as species in phylogenies or individuals in genealogies. Horizontal transfer is much more common in biogeography than in other systems, cases in which isolated fauna and flora begin to co-exist with new elements, so that the "age" of taxa in an area may differ. Most methods use the same term for all taxa occupying the same area. In logics, paraconsistency is the property of two equally denoted items being different, implying that logical consequence is not explosive, i.e., {A , ¬A} ? B. In biogeography this concept is applicable in the sense that the same area may have elements connected to different areas without denoting contradiction. Coexistence of different faunas and faunas is recognized in texts, but numerical analyses usually lack tools to discern biogeographical layers, thus avoiding unnecessary conflict between data. The inability of most methods to deal with "contradictory signals" results in loss or distortion of information. The Oriental diversity of Diptera is analyzed and its different layers are recognized. It includes elements: (1) of southeast Palearctic origin (i.e., connected to China or other Palearctic areas); (2) of Australian origin (with close relatives, e.g., in tropical Papua-New Guinea); (3) of tropical Gondwanan origin (with its relatives mainly in Africa); and (4) of tropical Laurasian origin (with most relatives extinct, and remnants present, e.g., in tropical Nearctic areas). Examples are given and implications are considered.

Keywords:*Biogeography, paraconsistency, biogeographical layers, Oriental Region*

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S110TH04

Systematics, Phylogeny and Zoogeography

The wings of butterflies: Active dispersal through time or passive movements?

Mikko Niklas Wahlberg
University of Turku, Finland

Recent advances in using molecular data, calibrated with fossil evidence, to estimate the times of divergence of lineages in almost any group of organisms has brought fresh understanding to where various organisms have originated and how they got to where they are today. I will review the state of knowledge in butterflies, a group that has long been speculated to have been influenced by the break up of Gondwana. Our work on the family Nymphalidae shows that most lineages are too young for vicariance to have played a major role in explaining the current distributions of species. In contrast, it is very clear that butterflies have actively dispersed, although it is apparent that successful colonizations of new areas happens rarely enough that clear phylogenetic patterns are retained.

Keywords:*dispersal, vicariance, Lepidoptera, timed phylogenies*

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The fossils within: Use of nuclear mitochondrial pseudogenes to understand historical biogeography

Hojun Song
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Inadvertent coamplification of nuclear mitochondrial pseudogenes (numts) is a serious problem in mitochondrial systematics, but numts can also be a valuable source of information because they represent ancient forms of mtDNA. In this presentation, I introduce a conceptual framework of numt accumulation, which states that in a given species there can be two types of numts, synaponumts and autaponumts, resulting from integration occurring respectively before and after a speciation event. In a given clade, a species that diverged early can only have its own autaponumts as well as synaponumts that were already present in the genome of the last common ancestor. A species that diverged more recently may, however, have many different synaponumts integrated at each different divergence as well as its own autaponumts. Therefore, it is possible to decipher the evolutionary history of a species based on the phylogenetic distribution of numts in a simultaneous analysis of numts and extant mtDNA. I empirically test this idea in the context of addressing a controversial question regarding the biogeography of the grasshopper genus Schistocerca Stal, based on numts of cytochrome c oxidase subunit I (COI) gene. I find that the empirical data can be adequately explained by the conceptual framework and that the phylogenetic distribution of COI numts reveals intricate evolutionary histories about past speciation events that are otherwise difficult to detect using conventional markers. This study demonstrates that numts provide interesting and powerful phylogenetic signal, much more than what extant mtDNA or nuclear gene sequences might be able to provide.

Keywords:*nuclear mitochondrial pseudogenes*

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The interface of biogeography and phylogeography in dune restricted insects in North America

Matthew H. Van Dam
UC Berkeley, U.S.A.

The sand dunes of Southwestern North America are a continental archipelago. Using probabilistic methods to explicitly test not only the pattern but also the divergence dates among taxa is a marked departure from previous parsimony based methods. I hope to synthesize the results from multiple unrelated taxa to test alternative dispersal patterns in this island system. I will also be addressing questions of species delimitation and how that plays an integral part in how we define biogeography and phylogeography.

Keywords:*Curculionidae, Mydidae, Rhaphidophoridae, comparative biogeography, divergence dating, Lagrange*

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S110TH07

Systematics, Phylogeny and Zoogeography

A history of bioregionalisation in Australia

Malte C. Ebach
University of New South Wales, Australia

The development of Australian biogeographical regionalisation since 1805 has been driven by colonial 19th-century exploration and by the late 20th-century biodiversity crisis. The intervening years reduced existing large scale regionalisations into smaller taxon specific areas of vegetation or endemism. However, large scale biotic biogeographical regionalisation were rediscovered during multi-disciplinary meetings and conferences, sparking short-term revivals, which have ended in constant revisions at smaller and smaller taxonomic scales. In 1995 and 1998, the Interim Biogeographic Regionalisation for Australia and the Integrated Marine and Coastal Regionalisation of Australia, Australian Commonwealth funded initiatives in order to "identify appropriate regionalisations to assess and plan for the protection of biological diversity", have respectively replaced 190 years of Australian biogeographical regionalisation. This talk looks at the rise and slow demise of biogeographical regionalisation in Australia in light of a fractured taxonomic biogeographical community.

Keywords:*Biogeography, Bioregionalisation, Australia*

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Global biogeographic patterns of grassland Auchenorrhyncha

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Patterns of grassland biogeography were inferred using 3 tribes of leafhoppers (Deltocephalini, Paralimnini, and Hecalini) and a family of planthoppers (Caliscelidae). Previous biogeographic study of this biome has been based on ungulates or other groups which have either limited distributions, or a small number of taxa. Although little is known about the relationships between closely related lineages of insects occurring on the various grasslands of the world available studies suggest a much more complex biogeographic history than that elucidated by ungulates. The lineages selected for this study however are hyperdiverse and globally distributed, although genera and species have localized distributions, allowing for fine scale patterns also to be drawn. In addition to biogeographic patterns we tested theories regarding host specialization, and comparing distributional relatedness patterns to grasses. Presented here are phylogenetic analysis of each lineage based on 3 genes, and a biogeographic analysis for each lineage. Preliminary phylogenetic results based on DNA sequence data indicate that biogeographic patterns of grassland leafhoppers are more complex than those of previously studied groups of grassland organisms, with multiple invasions of various grassland regions within each lineage. Along with phylogenetic and biogeographic results this study has conservation implications. Grasslands are rapidly being destroyed and identifications of regions possessing many unique lineages would allow these regions to be targeted for further protection.

Keywords:*biogeography, molecular phylogeny, leafhopper*

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S110TH09

Systematics, Phylogeny and Zoogeography

Historical biogeography of Australian true bugs

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Land bug (Insecta: Hemiptera: Geocorisae) diversification is inexorably linked to their interactions with seed plants. Our understanding of how these interactions impact on the distribution of these taxa is cursory. Australian land bugs are highly diverse, and a plethora of new studies enables their use as a model taxon for testing current theories of area relationships in Australia. Phylogenies and taxon areagrams are presented for monophyletic groups from the families Miridae, Tingidae, Rhyparochromidae, Piesmatidae and Coreidae. These demonstrate a major split between areas defined by the Great Dividing Range from those of arid and southwest Western Australia. These results are contrasted against independent studies for vertebrates and flowering plants, and a 'total evidence' areagram is presented for land bugs, vertebrates and flowering plants.

Keywords:*Historical biogeography, Heteroptera, Australia, host plants*

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Austral biogeography: Goodbye Gondwana and the Moa buoyancy hypothesis

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Our understanding of the biogeography of the southern end of the world has been revolutionized in recent years. The comfortable assumption that austral distributions can be explained by Gondwanan ancestry followed by vicariance has been overturned by evidence from studies estimating divergence times of key austral taxa using molecular data. A number of iconic gondwanan groups contain internal nodes that are too young to be explained by gondwanan vicariance, and suggest that long distance over-ocean dispersal has been an important process in shaping austral distributions. Our knowledge of these distributions and the processes that shape them will improve as we gain further insights into the distribution of extant and extinct austral lineages and improve divergence time estimation methods. A number of processes are likely to have affected the distribution of each austral lineage, and ecological factors will influence the probability of these processes. We discuss these issues using recent evidence from Diptera and Mecoptera.

Keywords:*biogeography, austral distributions, gondwana, Diptera, Tabanidae, Nannochoristidae, Mecoptera*

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Local variation, species, and biogeography of New Zealand beetles

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New Zealand is a well-known, model location for understanding biogeographic patterns and a review of the problems during on-going work to explain glacial refugia, endemism, island biogeogeography and species/population assembly is presented. One of the major challenges is the poor taxonomy of microcoleoptera which we have been rectified by 1) revising the species prior to genetic studies or 2) revising focal groups as genetic studies ensue. This has proven effective, but for hyperdiverse groups like Zopheridae (the 4th largest group of beetles in NZ with over 200 species), multiple new species and non-monophyletic genera have been encountered, which cannot be revised in a short time frame. Nevertheless, our studies have shown that offshore islands contain a mixture of old and new lineages, deep haplotype diversity on the mainland corresponds to glacial refugia and/or areas of high species richness, and biotic gaps are the result of glaciation extirpation and unequal rates of recolonization among species. Studies are now underway to investigate unusual genetic patterns that appear to be associated with living in the soil, aptery, and alpine distributions.

Keywords:*island biogeography, refugia, species concepts*

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Evolutionary and biogeographic patterns in African and Madagascan dung beetles (Scarabaeidae: Scarabaeinae)

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Three hypotheses are proposed and discussed: (i) Dung beetles evolved in Africa (ii) Dispersal into new areas as opposed to vicariance and (iii) Fungus as the ancestral food type with subsequent radiation onto vacant ecological space. Africa not only has some of the world's most spectacular mammals but also the richest dung beetle diversity. Although dung beetles have worldwide distribution, recent biogeographic and phylogenetic evidence supports the hypothesis that nine of the 11 tribes are unequivocally African in origin. A group once thought to have radiated either prior to continental fragmentation and possibly in response to the availability of dinosaur dung has been shown to be capable of widespread dispersal. The present-day genera, the endemic Helictopleurus and the three species of Scarabaeus, on Madagascar are comprised of lineages based on Miocene dispersers from Africa as opposed to evolving in situ after an ancient vicariant event. Their dispersal ability is substantiated by that of the genus Nesosisyphus, which is endemic to the relatively young (8 MYA) oceanic volcanic island of Mauritius thousands of kilometres from either Africa or India as potential sources of origin. Three genera from the earliest diverging lineages, Coptorhina, Delopleurus, and Frankenbergerius, are known to breed in and feed on fungus supporting the hypothesis of an ancestral feeding pattern on fungus with the change to mammalian dung being precipitated by both an increase in the amount of mammalian dung during the Tertiary and the ease of the switch between fungal microbes and those of dung.

Keywords:*Africa, Madagascar, dung beetles, dispersal, vicariance, fungus feeders*

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Morphology and biogeographic patterns of ground beetles in Far East countries (Coleoptera, Carabidae, Pterostichinae)

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Pterostichids species are largest sized group in ground beetles, they usually live in deep forest area and feed on worms or decayed organism. Among them, species of subgenus Nialoe of Pterostichus have differential distribution, they occurred only far eastern countries, Korea, Japan and China, all known species are endemic without overlapping with other countries. The subgenus Nialoe species are distinct sexual heteromorphy in last segment of abdomen. Special shape of projection in last sternum of abdomen is important external key for classification. In this subgenus, totally 51 species are known in the world (Bousquet, 2003). 41 species are distributed in Japan, and then Korea and china are recorded 9 and 1 species respectively. These species have not hind wing, so they must move only working, it is the one of the reasons of regional isolation and also is caused to raise the species diversity. Korea, Japan and China located closely, but they have quite different specific characteristics in protuberance's shapes of last abdominal segment in male. Comparative studies on subgenus Nialoe of Pterostichus by geography, morphology are provided.

Keywords:*Coleoptera, Pterostichus Nialoe biogeography*

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S110TH14

Systematics, Phylogeny and Zoogeography

Large-scale biogeography of Carabid beetles using phylobetadiversity methods

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Phylobetadiversity measures hold promise as an alternative means to view large-scale biogeographic patterns with a focus on the assembly of a fauna and its phylogenetic composition. Although these methods are now being used extensively in ecological studies and for relatively recent patterns, they have not been applied to geologically old patterns at the level of continents or globally. I will present results from an exploration of these methods applied to Carabid beetles with a Gondwanan distribution and across the islands of the Pacific. The study will be based on approximately 3000 species and a 500+ taxon phylogeny that will be the basis for the user compromise tree produced by Phylomatic. NRI and NTI measures will be used to look for the phylogenetic signature in Carabidae (Coleoptera) of "oceanic" vs. continents and "continental fragment" islands.

Keywords:*Gondwanan, Pacific Islands*

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Biogeographic patterns of Pacific Orthotylinae

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Biogeographic patterns in the southwest Pacific have long held interest for biologists, with a variety of theories proposed to explain taxic distribution and relationship of areas. The plant bug tribe Orthotylini (Insecta: Hemiptera: Miridae) comprises a high diversity of genera and species in this region of the Pacific, many of which are undescribed. Several of these taxa display characteristics, which are atypical of any of the suprageneric groupings of the Orthotylinae. We report on systematic revisions of several of these genera and analyse patterns of distribution in the southwest Pacific. These patterns are compared with current theories of area relationships for other components of the biota of the southwest Pacific.

Keywords:*Biogeography, Orthotylinae*

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Alticinae (Coleoptera: Chrysomelidae) diversity in Oaxaca, Mexico: A preliminary study

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These results are part of a preliminary study of the insect diversity of the southern Mexican state of Oaxaca. Presented are the results from fieldwork carried out in August-September 2010. Approximately 48 species in 25 genera of Alticinae (Coleoptera: Chrysomelidae) were collected. This is added to previously recorded data from the literature as well as records from several North American collection holdings.

Keywords:*Alticinae*

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Phylogeny and evolution of Chrysomeloidea (leaf beetles and long-horned beetles)

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The sister superfamilies Chrysomeloidea (leaf beetles and long-horned beetles) and Curculionoidea (weevils) together form the uniquely diverse clade Phytophaga. While the reciprocal monophyly of Chrysomeloidea and Curculionoidea is relatively well-established, many questions remain about the internal phylogenies of these superfamilies, and about timing and patterns of taxonomic and ecological diversification across Phytophaga. In this talk I will review the current status of our understanding of the phylogeny and evolution of Phytophaga with a special focus on the superfamily Chrysomeloidea. Additionally, I will share preliminary results from ongoing studies designed to clarify family- and subfamily-level relationships in Chrysomeloidea and timing and patterns of chrysomeloid diversification. Particular emphasis will be placed on the family Cerambycidae and the early-divergent subfamilies of Chrysomelidae.

Keywords:*Chrysomeloidea, phylogeny, evolution*

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S111TH03

Systematics, Phylogeny and Zoogeography

Secondary sexual characters in the Diabroticites (Chrysomelidae, Galerucinae)

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Secondary sexual characters (SSCs) result from structures that are modified in one of the genders, usually the male, considered to participate in non-sexual contact during copulatory behaviour. SSCs are regarded as both products of sexual selection, and a testimonial of the biodiversity. The relevance of SSCs throughout the history of chrysomelid beetles is remarkable. Among the species included in the subfamily Galerucinae senso strictu, the diversity of such characters is particularly impressive, with modifications reported as occurring in every tagma. The great specificity of these variations is often useful in the identification of male specimens at generic level, and the galerucine beetles of the section Diabroticites (tribe Luperini, subtribe Diabroticina) are no exception. From a total of 17 genera included in this group, 10 were first described based on at least one SSC, mostly found in the head. A review of the main SSCs reported in the Diabroticites is provided, with a discussion of its relevance for taxonomy. The evolutionary hypotheses stated for such a display of variation and its influence in the reconstruction of the phylogenetic history of this group are also presented.

Keywords:*sexual dimorphism, sexual selection, Diabrotica, morphology*

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Notes on the ecology of rolled leaf hispines (Chrysomelidae: Hispinae) at La Gamba (Costa Rica)

Michael Schmitt
Ernst-Moritz-Arndt-Universitat Greifswald, Germany

Michael Schmitt & Meike Frank Allgemeine & Systematische Zoologie, Ernst-Moritz-Arndt-Universitaet, D-17489 Greifswald, Germany, michael.schmitt@uni-greifswald.de During a field study at La Gamba (Golfito region, Costa Rica), a total of 322 adult hispine beetles of the genera Cephaloleia and Chelobasis have been found in rolled leaves of plants of 16 species of the order Zingiberales (families Costaceae, Heliconiaceae, Maranthaceae, and Zingioberaceae). Of these beetles, Cephaloleia belti was recorded from 11 host plant species, C. dilaticollis from 7, C. distincta from 6, Chelobasis bicolor from 4, C. championi from 3, and Chelobasis perplexa, C. histrionica, C. instabilis and C. trivittata from 2 each. Of the plant species, Heliconia imbricata had 7 beetle species in its leaf rolls, H. longiflora 6, H. rostrata and H. latispatha both had 5, Alpinia purpurata, Calathea lasiohylla, H. wagneri, H. stricta, and Calathea platystacha harboured 2 beetle species each, while on Costus pulverulentus, Calathea lutea, Calathea insignis, Costus barbatus, H. densiflora, H. vaginalis, and H. danielsana only hispines of one species were found. Cephaloleia belti occurred together with beetles of five other hispine species, whereas Cephaloleia trivittata never shared a leaf roll with another hispine species. The remaining beetle species aggregated with one to four other hispines. Adults of C. dilaticollis and C. championi were frequently seen to co-occur with the carabid Calophaena ligata in the same leaf roll without any sign of interspecific aggression. As they did not even interact in a perceivable manner, the nature of this association remains enigmatic.

Keywords:*Coleoptera, Chrysomelidae, rolled leaf hispines, host plant selectivity,*

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Biology of *Caryedon prosopidis* Arora (Coleopteran: Bruchidae) on acacia Arabica(Lam.) willd – A new host record

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Himachal Pradesh University, India

Bruchide constitutes a homogeneous family of seed beetles that attack seeds of wild and cultivated leguminous plants worldwide. Most economic species of bruchids belongs to six key genera viz. Acanthoscelides Bruchus Bruchidius, Callosobruchus, Caryedon and Zabrotes. Species belonging to Bruchidius and Caryedon are generally field bruchids that attack the seeds and pods of wild leguminous plants. Caryedon prosopidis Arora (Coleoptera: Bruchidae) has been reported initially from Prosopis juliflora (Swartz) DC but present findings have unveiled its infestations from pods and seeds of Acacia arabica (Lam.) Willd. Gravid female laid 19.5 + 1.4 eggs during dusk and down and incubation period lasted for 8.5 + 1.1 days. All larval instar were voracious feeders and their development was completed inside the host seed. Larval duration lasted for 29.7 + 2.2 days and last instar larva emerged out of seed by cutting a hole to pupate outside. Larvae undergo four moults before pupation and pupal stages lasted for 32.7 + 1.04 days inside the cocoon. Adult insects emerged out in 63.6 + 3.4 days after cutting a circular lid at one end of the cocoon and longevity of insect lasted for 30.0 + 1.67 day during favourable period.

Keywords:*Biology, Caryedon prosopidis, Prosopis juliflora, new record.*

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S111TH06

Systematics, Phylogeny and Zoogeography

Discerning pattern in the connection of Cassidinae (Coleoptera, Chrysomelidae) and host plants

Donald Windsor
Smithsonian Institution, Panama

Many of us record "biological data" such as host plant identity when we make collections. But what do we to do with this information? The construction of trophic webs is one option. Their construction distills a great deal of complexity into metrics and graphics which facilitate comparisons among taxa, guilds and communities. We present results from studies of Cassidinae leaf scrapers and leaf miners in Panama, Bolivia and elsewhere which reveal significant differences in the way some taxa within Chrysomelidae are connected to host plants.

Keywords:*Trophic Webs, Cassidinae, Chrysomelidae*

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Evolutionary history of gall-forming aphids of the tribe Eriosomatini (Aphididae: Eriosomatinae)

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Reconstruction of evolutionary transitions in insect-host associations on the inferred phylogenies is crucial for understanding the evolutionary histories of the insect group. We inferred phylogenetic relationships of the aphid tribe Eriosomatini, gall-formers on the ulmacean genera Ulmus and Zelkova, based on morphological characters. The evolution of the eriosomatine aphids is discussed in relation to the reconstructed aphid-host associations and information about host-plant phylogenies and fossil records. Phylogenetic analysis with equal character weighting led to hundreds of most-parsimonious trees, but the successive weighting of characters yielded three most-parsimonious trees, and the strict consensus of these was well resolved. Reconstruction of the aphid?host associations on the consensus tree indicated that the ancestral Eriosomatini were associated with Zelkova and that the association with Ulmus evolved twice independently. It is suggested that despite early diversification of the Eriosomatini on Zelkova species, Zelkova-associated eriosomatines had become extinct or survived as relict parthenogens on the secondary host since the late Tertiary. In contrast, two large genera in the Eriosomatini, Eriosoma and Tetraneura, are associated with the largest elm section Ulmus whose elements are distributed widely in Eurasia, including boreal regions. Therefore, the available evidence suggests that the present species diversity and distribution patterns of the eriosomatines have been largely affected by the diversification and extinction of their host plants during the late Tertiary and Quaternary.

Keywords:*distribution, diversification, insect?host associations, relicts, species diversity, Ulmus, Zelkova*

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Patterns of diversification of the conifer feeding-aphid genus *Cinara* (Lachnidae): Biogeographical history and evolution of host plant association

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Cinara is a large genus of Aphididae, with 222 species, specialized on Gymnosperms, mainly on the genus Pinus. It is commonly thought that the conifer-feeding genus Cinara, had an angiosperm-feeding ancestor and secondarily acquired its coniferous hosts. This shift appears to be associated with a recent successful radiation onto a young conifer family (Pinaceae). Speciation within this genus is said to be mostly driven by host switch. The aims of our study are: (i) to produce a robust molecular phylogeny of the genus and explore the validity of the taxonomic subdivisions, (ii) to use this reconstruction to study patterns of diversification in this genus, we will investigate the respective roles of host-plant association, biogeographical history and aphid feeding sites. Using more than 200 specimens, representing 58 species, we built a molecular phylogeny with 6 DNA fragments (mitochondrial, nuclear and Buchnera aphidicola). Our phylogenetic trees confirm the monophyly of all described species however it shows that some species clearly encompass several genetic clades. We therefore used a DNA species delimitation method to produce a species tree. The mapping of host plant association on this reconstruction reveal several major switches between genera Pinus and Picea during Cinara's evolution. Biogeographical reconstructions also suggest several dispersal events between Europe and North America. Evolutionary transitions in Cinara's feeding sites have also accompanied Cinara's speciation events. We discuss alternative scenarios for the rapid diversification of this aphid genus.

Keywords:*Cinara, Lachnidae, biogeography, phylogeny*

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S112W03

Systematics, Phylogeny and Zoogeography

Population genetic structure of the cotton-melon aphid, *Aphis gossypii* Glover, in primary and secondary hosts

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Population diversification due to host-specialized populations in the polyphagous cotton-melon aphid, Aphis gossypii, is remarkable among all aphid species. Aphis gossypii utilizes 148 plant species as host plants in which those host-varied populations possibly speciated or genetically isolated into several populations. These hosts are classified into primary (winter) and secondary (summer) hosts; the former specialized for mating and oviposition, and the latter for proliferation by parthenogenetic reproduction. It is known that some strains of A. gossypii can alternate primary and secondary hosts in the temperate region. Because secondary hosts diverse further than primary hosts, it is suggestive that genetic diversity increase when different populations isolated by secondary hosts return to interbreed there. In this study, we aim to confirm how the population isolations were correlated with host-varied, geographic, or host-preferred factors, and to clarify the pattern of population-level diversification based on population genetics. We performed population genetics using the demes alternating between winter (primary) and summer (secondary) hosts in the cotton-melon aphids. We analyzed population genetic structure between 570 aphids collected from 41 plant species of primary and secondary, mostly wild, hosts using 9 microsatellites loci. As results, population structure revealed that several genetic affinities in use of some secondary and primary hosts are detected. Host preference in secondary host is higher than that in primary host, and woody plants share same genetic structure. We will discuss whether these aphid species speciated by the related mechanisms such as host alternation and loss of primary host.

Keywords:*Aphis, gossypii, population genetics, host race,*

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S112W04

Systematics, Phylogeny and Zoogeography

Review COI seqeunces of the Aphididae, and the necessity of developing new molecular markers for aphid species identification

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Several DNA barcode studies were conducted on the family Aphididae, reporting the usefulness of the mitochondrial COI gene as DNA barcodes in identifying aphid species. However, it sometimes caused misidentifications mainly due to low interspecific genetic divergences among some aphid species. In this study, to determine useful molecular markers to improve this problem, 1,257 sequences of the ten genes (COII, CytB, ATP6, lrRNA, srRNA, ITS1, ITS2, EF1a, 18S, and 28S) were examined along with 1,019 sequences of COI gene. Our analyses showed that the ATP6 gene revealed higher genetic divergences than those of COI at conspecific and congeneric taxonomic levels. The possibility of the ATP6 gene as an alternative molecular marker was explored by using it to identify thirty aphid species, confirming that ATP6 gene is suitable marker to improve on the rate of misidentifications.

Keywords:*Aphididae, ATP6, COI, molecular species identification*

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Reconstruction of subtribal concept in the tribe Macrosiphini (Hemiptera: Aphididae) based on molecular data

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The tribe Macrosiphini is one of the largest group within family Aphididae, comprising near 50% of the 5000 described species. According to previous studies of aphids, the phylogenetic relationship of Macrosiphini has been controversial and still ambiguous at the subtribal level. With the results of comprehensive molecular phylogenetic study by Bayesian phylogeny (BP) and Maximum-likelihood (ML) using combined data sets of molecular sequences (cytochrome oxidase I (COI), tRNA-leucicne+cyrochrome oxidase II (tRNA+COII), 16S ribosomal RNA (16S rRNA) and Elongation factor-1 alpha gene (EF1α)), we reconstruct the subtribal groups for the tribe Macrosiphini.

Keywords:*Aphididae, Macrosiphini, COI, tRNA+COII, 16S rRNA, EF1α.*

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Phylogeography, life cycle evolution and differentiation of sibling species in the leaf-curling plum aphid, *Brachycaudus helichrysi* (Hemiptera : Aphididae).

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Brachycaudus helichrysi is a worldwide polyphagous aphid pest that seriously damages its primary hosts (Prunus spp.) and the various cultivated plants among its secondary hosts (e.g. sunflower, Chrysantemum). In a recent phylogenetic study based on worldwide sampling, we have shown that this species is actually an amalgamation of two sibling taxa, B. helichrysi H1 and B. helichrysi H2. This study based on mitochondrial, nuclear and Buchnera aphidicola DNA markers revealed that these two taxa display levels of genetic divergence as great as those generally found between sister species in the Brachycaudus genus, suggesting that they correspond to two distinct species. Further investigations, based on microsatellites data combined with ecological information suggest that these two species have two very different evolutionary histories. B. helichrysi H1 exhibits a typical signature of a heteroecious life cycle on plum trees, and we demonstrate the existence of distinct geographic genetic clusters within this "species". By contrast B. helichrysi H2 gather two types of lineages. First, we demonstrate the existence of several persistent clonal lineages, distributed worldwide and highly polyphagous. Second, we found a heteroecious lineage that use peach trees as primary hosts and has so far only been found in found in Asia. All B. helichrysi lineages can be found in mixed colonies on herbaceous hosts all around the world. At some locations, individuals exhibit introgressed genotypes between B. helichrysi H1 and H2. We discuss how life cycle evolution, leading to reproductive barriers, could explain the ongoing speciation in the B. helichrysi species complex.

Keywords:*life cycle evolution, cryptic species, reproductive mode, asexuality, phylogeography*

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S112W07

Systematics, Phylogeny and Zoogeography

Evolution of asexuality in the pea aphid, *Acyrthosiphum pisum*

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Many aphid species include asexual lineages that are often distributed in the southern region of the distribution range. Asexual lineages could originate through spontaneous loss of sexuality arising from environmental conditions or mutation, or hybridization between closely related species or host races. In the present study, we examined how asexual lineages of A. pisum originated in East Asia by using genetic and phylogenetic methods. In northern regions of Japanese islands, sexual host races were associated with Vicia cracca, Vicia angustifolia, alfalfa and clover. Asexual lineages of A. pisum were found widely on Vicia angustifolia in the main island of Japan, where each local population consisted of a few asexual clones with different genotypes. Thus, genetic diversity in asexual population was markedly limited. Analysis with microsatellites indicated that the major clones (super clones) often had considerably different constitution of alleles from each other. Phylogeny constructed using microsatellites showed that asexual populations on Vicia angustifolia did not constitute a monophyletic group with sexual populations on the same plant. Cloning and sequencing of EF1α alleles from each asexual individual indicated that a part of these individuals contained an allele that was specific to sexual populations on Vicia cracca and a different allele that was specific to sexual populations on Vicia angustifolia. Therefore, our results suggest that in A. pisum, the origin of asexual lineages is partly ascribed to hybridization between different sexual host races, as indicated for Rhopalosiphum padi by Delmotte et al. (2003).

Keywords:*pea aphid, asexual, hybridization*

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S112W08

Systematics, Phylogeny and Zoogeography

Host-associated differentiation among host-sharing aphids in the gossypii group

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Insect herbivores, especially aphids, are closely related with their host plants, which are crucial for their life as the only habitat as well as food resources. Current species-richness of aphids is highly associated with their complex life cycles including host alternation and cyclical parthenogenesis on various host plants. Host alternation is considered one of the most critical factors for their differentiation driven by loss of primary host. Interestingly, both monoecious and heteroecious aphids exist in the gossypii group belonging to the most species-rich genus Aphis. In this study, we examined genetic structure of four heteroecious species, Aphis gossypii Glover, A. glycines Matsumura, A. sp. ex Rhamnus, and A. punicae Passerini, in gossypii group using mitochondrial COI sequence and 9 cross-species-amplifiable microsatellite loci. These four closely-related species occur together on several primary and/or secondary hosts: Rhamnus, Rubia, Punica and Commelina. We also identified four genetically differentiated clusters associated with Hibiscus, Rhamnus and several secondary hosts within A. gossypii. Majority of secondary-host-associated populations were affinitive with the Hibiscus-associated group, whereas several host races collected from Sedum and Ixeris were considerably differentiated with Hibiscus-associated group. These high divergences might be caused by loss of primary host. Some incongruities between COI and microsatellite genotypes, and our confirmation of inter-specific copulation suggest that there would be a continuous gene flow between host-sharing species of the gossypii group in same primary host as a hybrid zone.

Keywords:*ecological speciation, gossypii group, host alternation, hybridization, population genetics*

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S113F02

Systematics, Phylogeny and Zoogeography

Phylogeny and social evolution of the Halictidae

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The bee family Halictidae is one of the most interesting groups of insects from the perspective of social evolution. More behavioural diversity is present in the halictid bees than any other comparable taxon. The halictid bees were once thought to have evolved eusociality 'many' times. New phylogenetic results - focussed on the tribe Halictini, which includes the most behaviourally diverse genera - and model-based ancestral state reconstruction suggest fewer and more ancient origins of eusociality than previously believed.

Keywords:*Hymenoptera Apoidea bees*

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S113F03

Systematics, Phylogeny and Zoogeography

Phylogeny and macroevolution of colletid bees (Hymenoptera: Colletidae)

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Colletid bees have been viewed as a prime example of an ancient group whose Gondwanan origin could have preceded the complete breakup of Africa, Antarctica, Australia and South America, because modern lineages of this family occur primarily in southern continents. The diversification of colletid bees was investigated to elucidate processes that may have contributed for the present diversity as well as southern disjunctions. A data set comprising four nuclear genes of a broad sample of Colletidae was assembled and Bayesian inference analyses to estimate the phylogenetic relationships as well as divergence times. Lineage-through-time analyses were used to explore the tempo of radiations of Colletidae and its context in the biogeographical history of these bees. Initial diversification of Colletidae took place at the Late Cretaceous (≥ 70 Ma). Several lineage exchanges between Australia and South America through Antarctica during the Late Cretaceous through the Late Eocene could explain the disjunctions observed between colletid lineages today. Higher diversification rates in the later period of colletid history were detected. From these findings, it can be concluded that biogeography and macro-evolutionary history of colletid bees is explainable by a combination of Cenozoic vicariance and palaeoclimate changes during the Neogene. The austral connection followed by breakup of Antarctica, Australia, and South America resulted in disjunct sister-lineages in the latter two continents. Increased biome aridification coupled with floristic diversification in the southern continents during the Neogene may have contributed to the high rates of cladogenesis of these bees in the last 25-30 Myr.

Keywords:*Apoidea, Gondwana, biogeography, Antarctica, rates of diversification, palaeoclimatology, vicariance*

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S113F04

Systematics, Phylogeny and Zoogeography

Recurrent evolution of dependent colony foundation in the aculeata

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Eusocial insects are often championed as icons of cooperation and specialization, yet in the majority of species new colonies are initiated by a solitary queen acting alone. In many bees, ants and wasps, selection against this solitary phase has lead to the recurrent evolution of dependent colony foundation (DCF; also known as fission, swarming and budding), whereby new colonies are founded by groups of individual nestmates. While this strategy is widespread, and characteristic of some of the most ecologically and economically important species (eg: honeybees, stingless bees, army ants), it remains poorly understood. Furthermore, adoption of dependent colony foundation has implications for diverse phenomena including population genetics, dispersal, reproductive investment, invasiveness, morphology and ecological flexibility. In this presentation I review the phylogenetic distribution of DCF across the aculeata, and summarise data from some recent studies on ants. DCF is both more widespread and more diverse than generally acknowledged, and more studies focusing on DCF are needed.

Keywords:*Hymenoptera, fission, budding, swarming*

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S113F05

Systematics, Phylogeny and Zoogeography

Radiation of the allodapine bees since the Eocene

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Allodapine bees are largely restricted to the southern Old World and provide an ideal group for exploring Southern Hemisphere historical biogeography and the effects of climate change associated with establishment of the Circumpolar Current. The Allodapini originated in Africa and there have been multiple dispersals to Asia, the Middle East, Madagascar and Australia. Here we show that radiations in Australia and Africa show very different historical patterns od diversification. The Australian radiation is marked by a strong response to aridification in the Miocene, probably the result of increased allopatric speciation as cooler habitats became fragmented, but such a response is absent in Africa. Our results indicate very different responses to climate change at a continental level and suggest that future responses of even closely related taxa are likely to vary enormously among southern landmasses.

Keywords:*bees, phylogenetics, radiations, dispersal, Allodapini, Apidae*

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S113F06

Systematics, Phylogeny and Zoogeography

Radiation of bees in the South West Pacific - Dispersals and rapid radiations

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Although bees form a key pollinator suite for flowering plants, very few studies have examined the evolutionary radiation of non-domesticated bees in human time-scales. This is surprising given the importance of bees for crop pollination and the effect of humans in transforming ecosystems via agriculture. Here we explore the radiation of halictine bees in Fiji following their post-Miocene colonization of these islands. Our mtDNA barcode data indicate the existence of several 'deep' clades whose divergences are close to the crown node, along with another highly derived 'broom head' clade with very high haplotype diversity mostly limited to low-lying agricultural regions. Whilst this derived clade is very abundant, the more basal clades were relatively rare. Although nearly all haplotype diversity in Fijian Homalictus comprises synonymous substitutions, a small number of amino acid changes are associated with the major clades, including the hyper-diverse clade. Analyses of haplotype lineage accumulation show a steep increase in selectively neutral COI haplotypes corresponding to the emergence of this 'broom head' clade. We explore three possible scenarios for this dramatic increase: i) a key change in adaptedness to the environment, ii) large-scale extinction event, or iii) a dramatic increase in suitable habitats leading to rapid population expansion. Using estimated mutation rates of mitochondrial DNA in other invertebrates, we argue that this increase may coincide with a period of agricultural intensification in populated coastal areas. Our results indicate that non-domesticated bee species may have responded strongly to human activities, and we discuss the implications of this finding.

Keywords:*Fiji, Halictinae, halictine, extinction, adaptation, agricultural intensification, DNA Barcode, COI*

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S113F07

Systematics, Phylogeny and Zoogeography

Phylogenetic perspective of a nocturnal niche invasion

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Comprehending how bees invade, or create, novel niches greatly enhances our understanding of the process of evolution. Behaviour is often the driving force of change, while genetic modifications permit adaptations that take advantage of these novel behaviours, and allow us to track their evolution. Most bees forage in bright sunshine, but some linegaes visit flowers in very dim-light, or are fully nocturnal; which is proposed as a response to competition, predation and parasitism. The sweat bee tribe Augochlorini contains the most diverse lineage of dim-light foraging bees. Their phylogenetic history, based on three nuclear genes (2K nucleotides), suggest a single origin (approx. 22 Mya), with at least one reversion to diurnal foraging from a nocturnal ancestor. Analysis of the longwavelength (green) opsin gene suggest a functional mutation of an amino acid - at the site where the chromphore binds to the opsin protein, that theoretically enables vision at alternate wavelengths. The implications of these findings are discussed in light of recent discoveries in opsin biology and the functional link between opsin evolution and circadian rhythm. The phylogenetic history of closely related species that inhabit alternate light environments provide the ideal system to explore these connections. Circadian rhythm in castes of highly social insects show periodicity dependent on behavioural reportoire, and the relationships between circadian clock evolution and social castes is discussed.

Keywords:*bee evolution*

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S113F08

Systematics, Phylogeny and Zoogeography

Evolution of sociality in the apoid wasp *Microstigmus nigrophthalmus*

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The order Hymenoptera is rich in origins of social behaviour, yet several of these remain very little studied. Microstigmus is a neotropical genus of apoid wasps which represents an origin of sociality independent of vespoids. Until recently, studies of colony genetic structure in this clade were limited to one species, M. comes. Combining experimental manipulation, behavioural observations and hypervariable genetic markers, we investigated the social and genetic structure of M. nigrophthalmus, a species from the Atlantic forest habitat of Brazil, known to nest in groups. We found that M. nigrophthalmus exhibits an unusual social system that differs from both M. comes and vespoids: there is cooperative brood care, and several females may share reproduction in the same nest, but it appears to be these reproductive females that perform most of the foraging effort. We also investigated the potential benefits to sociality in this species. Mean relatedness between female nestmates was high (0.49), so that females obtain indirect benefits through helping, but relatedness was very variable and could be very low in some nests. Furthermore, experimental removal of reproductive females from the nest revealed that orphaned brood continued to be cared for by surviving nestmates, indicating that a group-nesting female could benefit from the insurance that her offspring will continue to be raised to adulthood in the event of her death (Assured Fitness Returns).

Keywords:*Microstigmus, Crabronidae, Assured Fitness Returns, Eusociality*

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S113F09

Systematics, Phylogeny and Zoogeography

Antiquity and evolutionary history of sociality in carpenter bees

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Understanding how advanced social organization has evolved comprises one of the key puzzles in evolutionary biology. We know that once this kind of social complexity evolves it can be enormously successful, but we also know that it has evolved very, very rarely. The paradox of why such an enormously successful strategy has evolved so rarely is one of the major questions facing evolutionary biology. Resolving this critical paradox is very difficult because most of the socially complex species have few surviving relatives that are solitary, so we are unable to examine the evolutionary stages leading from simple to complex organization. The bee subfamily Xylocopinae contains four extant tribes. Three of these, the Allodapini, Xylocopini and Ceratinini, have been extensively used to investigate social evolution. The fourth tribe, Manueliini contains species whose biology has been largely unknown until very recently. It is important to understand the phylogenetic position of Manueliini so we can explore, whether sociality is plesiomorphic for the Xylocopinae or whether it evolved multiple times in this subfamily. Using phylogenetic inference and nesting biology studies on 34 species across all four tribes reveals that a weak form of sociality is ancestral for the Xylocopinae, and was in place for over 90 million years. In this subfamily the earliest societies did not entail a foraging worker caste, but instead comprised females sharing a nest with supersedure of dominance.

Keywords:*carpenter bees*

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Systematics, Phylogeny and Zoogeography

Adephagan phylogeny based on sequence data from the BToL project

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I will present preliminary results of analyses of DNA sequence data from four loci (ArgK, CAD, wg 28S) for Adephaga (Coleoptera) with an emphasis on carabid beetles. Implications for the evolution of aquatic life history, placement of classically problematic taxa and well-supported and novel phylogenetic relationships will be discussed. I will present preliminary results of analyses

Keywords:*Adephaga, Carabidae*

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Evolutionary patterns of hind wing morphology in scarabs (Coleoptera: Scarabaeoidea) based on the extant and extinct evidences

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The shape of wings can be a good predictor of adaptations to different selective pressures and the value of wing features in taxonomy and phylogeny has long been recognized. Previous studies have analyzed the evolution of wing shape within a single or very few species, or by comparing only a few wing traits. No study has analyzed wing shape evolution of a large number of species, or quantitatively compared morphological variation of wings with proposed phylogenetic relationships. Two of our studies examined the morphological variation of hind wings based on 19 landmarks, 119 morphological characters, and 81 beetle species. Another two studies published two new fossil families of Scarabaeoidea, based on the geometric morphometrics analysis of hind wings and cladistic evidences. In our investigations of the hind wing evolution of scarabs, we use geometric morphometrics combined with a cladistic approach. The tools of geometric shape analysis have a tremendous advantage in this application: it does not only offer precise and accurate descriptions, but also serves the equally important purposes of visualization, interpretation and communication of results.

Keywords:*scarab, wings, evolution, geometric morphometrics*

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Phylogenetic relationships of Cleroidea

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Members of Cleroidea (Cucujiformia) were once included in a broadly-defined Clavicornia along with tenebrionoids and cucujoids, while those having soft bodies like Melyridae were placed in Malacodermata with cantharoids and other taxa. Now, Cleroidea consists of 10 families and is usually considered monophyletic based on the absence of a mandibular mola in adults and larvae, basal mandibular processes (lacinia mobilis) present in larvae, and a pedunculate seta present on the mala. Phylogenetic relationships among the family-groups of cleroids is far from settled, and in the latest phylogeny of Lawrence et al. (2011), the cleroids were polyphyletic, made so by the placements of some members of the Trogossitidae outside Cleroidea as sister taxa to two separate cucujoid groups. Moreover, the remaining trogossitids contained within the superfamily formed a rather messy grade, indicating that a more detailed look at Trogossitidae is required to better understand character distributions and paraphyly with regard to the entire Cucujiformia. A first step is an appraisal of trogossitids and we present a local phylogenetic study of cleroids to address the issue of trogossitid polyphyly. We derive a more restricted data set from Lawrence et al. (2011) composed of all cleroid families and selected out-taxa from major groups of Cucujiformia. We also include observations made from confirmed larvae of the gondwanan trogossitid subfamily Rentoniinae. With the addition of new data, we confirm Trogossitidae paraphyly, as well as non-monophyly of other cleroid families. We advocate a reclassification of the Trogossitidae with the recognition of at least one new family.

Keywords:*Trogossitidae, Cleroidea, Phylogeny*

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The Phylogeny of Tenebrionoidea (Cucujiformia) inferred from molecular data

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The Tenebrionoidea are a morphologically and ecologically diverse lineage of over 35,000 species of beetles. We report recent phylogenetic findings on the relationships within this superfamily. Three nuclear protein-coding genes (Argk, CAD, wg) and one ribosomal gene (28s) were sampled across 120 taxa representing all 28 currently recognized families in the Tenebrionoidea. Diverse lineages of Cucujiformes were also sampled to examine the superfamily's monophyly. Likelihood analyses supports the monophyly of the Tenebrionoidea with the inclusion of the Lymexylidae and also suggest the need for redefining the limits of some of the families (e.g. Tenebrionidae, Mycteridae). We also discuss the major clades recovered by our datasets and compare these to existing phylogenetics hypotheses for the group.

Keywords:*Tenebrionoidea, Coleoptera, molecular phylogeny, nuclear protein-coding genes*

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S114F05

Systematics, Phylogeny and Zoogeography

Phylogenetics of weevils (superfamily Curculionoidea)

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The monophyly of the Curculionoidea (weevils) is supported by both molecular and morphological evidence. Recent phylogenetic studies also indicate that the Chrysomeloidea (leaf beetles and long-horned beetles) are their closest relatives, forming together a clade "Phytophaga" within the Coleoptera Cucujiformia. The present contribution will focus on the evolutionary history of the Curculionoidea, with approximately 62,000 described extant species, 80 % of which belong to the family Curculionidae. Being so extraordinarily speciose, the phylogenetic systematics of weevils remains most challenging. An updated molecular phylogeny of weevils is presented, based on analysis of DNA sequences from two nuclear (entire18S rRNA and segments D2-D3 of 28S rRNA) and two mitochondrial (domains IV-V of 16S rRNA and COI-3' section) markers, from a worldwide sample now expanded to include approximately 200 weevil genera and several outgroups. The gene fragments chosen are commonly sequenced for phylogenetic studies of Coleoptera and other insects. Here, the alignment of the sequences from ribosomal genes (18S, 28S, and16S) was performed according to secondary structural information, a method that uses a biological criterion (covariation) for assigning positional nucleotide homology. The value of incorporating structural information in the process of alignment is highlighted. Besides recovery of nodes consistent with major weevil lineagespreviously defined by adult and larval synapomorphies, molecular evidence from this study contributes to resolving controversial areas of weevil phylogeny, for example, the "platypodine problem", and to helping identify main clades within the megadiverse Curculionidae. A succinct account is given of the major groups, summarizing their diversity, fossil information, and biology.

Keywords:*Curculionoidea*

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Nomenclature of family-group and genus-group names in Coleoptera

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The most recent International Code of Zoological Nomenclature (ICZN 1999) included a new article entitled List of Available Names in Zoology (LAN). The goals of this Article are to promote nomenclatural stability by summarizing data on all available names, in a particular group and at a particular rank, by paring away dubious names in the literature and ensuring that such list is thoroughly vetted by experts. The Commission has received two submissions for Parts of the LAN to date (family-group names in Aphidoidea and species-group names in Rotifera) but none have been adopted yet. Coleopterists recently synthesized data on extant and fossil Coleoptera family-group names for the first time (Bouchard et al. 2011). Nearly 4500 suprageneric names were determined to available. For each name, the original spelling, author, year of publication, page number, correct stem and type genus were included. Genus-group names in Coleoptera have attracted much more attention from authors interested in nomenclature, usually specialists working on one or a small number of families. The results of these efforts have been the production of numerous catalogues sometimes with broadly variable interpretations of nomenclatural acts for similar problems in different groups. In our presentation, we will outline our plans for submitting a Part of the LAN relating to family-group names in Coleoptera in the near future. In addition we will discuss the potential establishment of a major collaborative effort with the ultimate goal to one day submit a Part of the LAN relating to genus-group names in Coleoptera.

Keywords:*Coleoptera, nomenclature*

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Reconstructing the Beetle Tree of Life

Duane D. McKenna[1], Alex Wild[2], Kojun Kanda[3], David Maddison[4], Brian D. Farrell[5]
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The lack of a relatively comprehensive and well-supported phylogeny for beetles (Coleoptera) has left us with many lingering questions about beetle relationships and has hampered efforts to reconstruct timing and patterns of beetle evolution. In this talk I will present an overview of beetle relationships based on analyses of nuclear DNA sequence data from the Beetle Tree of Life Project. I will discuss relationships across the order Coleoptera, but my presentation will be focused on the extraordinarily diverse suborder Polyphaga. Within Polyphaga I will discuss relationships among lower-level groups, e.g., series, superfamilies and families. Additionally, I will highlight some success stories while also noting groups whose interrelationships remain unclear.

Keywords:*Coleoptera, phylogeny, macroevolution*

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S114F08

Systematics, Phylogeny and Zoogeography

A summary tree of Coleoptera from available molecular data

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The growing database of publicly available DNA sequences provides an increasingly detailed picture of the phylogenetic relationships of Coleoptera. We present a single tree of >8000 species based on the most widely used genetic loci. Our recent efforts aimed at 'gap filling' of unsampled lineages and missing loci has greatly reduced the inconsistencies of previous compilation from GenBank sequences. The resulting tree is a now satisfactory framework for placing incoming new sequences, including environmental samples, and permits reliable placement of unknown samples based on a range of loci. Already many incompletely identified sequences complicate tree building and concatenation of sequence data across loci, but we have developed methods that minimize these problems. Besides methodological aspects of building very large trees from data mining, the talk will also discuss the increasingly well supported basal relationships of Coleoptera.

Keywords:*Coleoptera, bioinformatics, large-scale phylogenetics*

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S115F01

Systematics, Phylogeny and Zoogeography

Origin of views on origins of hexapods

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To Linnaeus all arthropods were 'Insecta', with the non-pterygotes (including some secondarily wingless pterygotes) grouped into the order 'Aptera'. A refined systematization of the 'Aptera' was initiated by his foremost entomologist student Fabricius and continued by Latreille, who eventually excluded crustaceans, arachnids and finally myriapods from 'Insecta'. A concept of 'origin' evidently required evolutionary thinking, and Haeckel depicted hexapods on a 'phylogenetic tree', sharing with the myriapods a branch from the 'Tracheata' stem, which in turn is shared with the arachnids, and joins the Malacostraca stem before that of the entomostracans. Symphyla were so named (Ryder 1880) exactly to emphasize their "singular combination of myriapodous, insectan and thysanurous characters"; a concept of symphylans being particularly relevant for elucidating hexapod origins remained advocated by outstanding 20th century writers. The distinction between primarily and secondarily wingless hexapods was introduced first by Brauer and Lubbock, but Brauer's fellow-countryman Handlirsch later derived the pterygotes directly from tilobites, not from apterous hexapods. While the 'Tracheata'/'Atelocerata' concept had the upper hand throughout most of the 20th century, close Crustacea+Hexapoda affinities were not without advocates during its first half. Even hexapod non-monophyly was given serious consideration, with Collembola being outside a lineage comprising myriapods and other hexapods (Imms), or with a taxon 'Myocerata' comprising entognathans+myriapods being opposed to the Insecta s.str. ('Amyocerata', Remington). However, apart from the 'Mantonian interlude' (concepts of otherwise unimagined polyphyly of arthropod lineages), a monophyletic Entognatha+Insecta being the sister group of myriapods, or a subgroup thereof, appeared a near-consensus model until the 1990s.

Keywords:*Evolution, history*

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S115F02

Systematics, Phylogeny and Zoogeography

How did the first hexapod look like: Status quo and problems in reconstructing the ground pattern of Hexapoda

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Our knowledge on the ground pattern of the morphology of Hexapoda is still quite limited for several reasons. Despite the high agreement that hexapods are nested within paraphyletic crustaceans, the precise hexapod sister group within Pancrustacea is still hotly debated. Many older attempts to reconstruct the ground pattern of Hexapoda mostly were based on a hypothesized sister group relationship with myriapods, or a subgroup of the latter. Other attempts to reconstruct the first hexapod are not based on outgroup comparison, but on sometimes not explicitly mentioned assumptions regarding the nature of character evolution. Additionally, the reconstructed ground pattern often only reflects the situation in Archaeognatha and Zygentoma, while the entognathous groups are implicitly assumed to secondarily display more derived conditions. Selected character systems will be chosen to highlight problems in the ground pattern reconstruction. Partly character polarization based on empirical data allows for no unambiguous reconstruction of the ground pattern. In other instances, however, reevaluation of character system results in phylogenetically informative characters, with strong impact on our insights into both character evolution and phylogeny.

Keywords:*character polarization, ground pattern, Entognatha*

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Exploring morphological variation in apterygotes: Consequences of taxon sampling on ancestral character state reconstructions

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Previous attempts to infer the early evolution of hexapods from comparative morphology are based on only a few, more well-known apterygote exemplars. In recent years some lesser-known taxa have been included into cladistic analyses and characters of internal anatomy have been explored to cover phenotypic variation more comprehensively. The current taxon sampling especially includes projapygoids and non-japygid Dicellurata among Diplura, as well as the Nicoletiidae, Protrinemuridae, and Maindroniidae among Zygentoma. Based on the new sampling the ancestral character states for each of the apterygote orders are revised and used for discussion of the basal splits among hexapods. Current insights reveal a remarkable degree of homoplastic transformations during the early evolution of hexapods, including convergent gain of characters related to terrestrial life habits. The phylogenetic resolution of the Diplura among apterygotes has the most drastic impact on our understanding of the hexapod ground pattern, whereas the contribution of extinct apterygote taxa presently remains ambiguous. Outgroup comparison with crustaceans still is a major deficiency in comparative hexapod morphology. In order to contribute to a clarification of the hexapod origin, one imminent task is to reevaluate many anatomical characters that are presently considered as mutually inapplicable in analyses of crustacean and hexapod phylogeny.

Keywords:*Evolutionary morphology*

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S115F04

Systematics, Phylogeny and Zoogeography

Early splitting of Hexapoda reviewed from the comparative embryology

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Phylogenetic relationships of hexapod basal clades have been greatly debated from multiple sources, but are still not satisfactorily resolved. For example, doubt has been cast on the reality of the Entognatha-Ectognatha system, especially regarding the status of Entognatha. In the past few decades, much embryological knowledge concerning Apterygota has been accumulated. I discuss the early splitting of hexapod basal clades and phylogenetically reconstruct them based on recent progress of apterygote comaprative embryology. Comparative embryological analyses on the basal clades of Hexapoda demonstrate an anagenetical transition of the functional specialization between embryonic membranes and embryo proper, leading to the acquisition of elaborated structures derived from the embryonic membranes. Hereby, the basal splitting of Hexapoda may be formulated as: Ellipura (= Protura + Collembola) + (Diplura + Ectognatha (= Archaeognatha + Dicondylia (= Zygentoma + Pterygota))). Ellipura is supported, but Entognatha is dismissed. Comparison of entognathy formations among three "entognathan" orders reveals a close resemblance between Protura and Collembola but a marked contrast between Diplura and Ellipura (the detail is given in the talk by Tomizuka et al.). Also as for other embryological features such as the manner of germ band (= embryo) formation and that of the primary dorsal organ, the congruency between Protura and Collembola and the discrepancy between Ellipura and Dilura are revealed. On the other hand, it should be noticed that Diplura and Ectognatha share the possession of a second embryonic membrane or the amnion, which may be regarded as being an embryological synapomorphy of Diplura and Ectognatha.

Keywords:*Comparative embryology, Apterygota, Protura, Collembola, Diplura, Archaeognatha, Phylogeny*

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Entognathy formations of three entognathan constituents, Protura, Collembola and Diplura

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Recently, "Entognatha-Ectognatha System" has been challenged, and doubt has been cast on the interordinal relationships within and monophyly of Entognatha from multiple sources. Entognathy has been regarded as a most reliable "autapomorphy" of Entognatha. We compare the entognathy formations of Protura, Collembola and Diplura, examine the homology of their entognathies, and discuss the interordinal relationships and monophyly of Entognatha. Protura and Collembola are revealed to closely resemble each other in major points of entognathy formation, such as concerning: 1) the origin of mouth folds, which are formed by the extension and fusion of terga of intercalary and three gnathal segments, and 2) origin of the postgnathal region of entognathy, which is derived from labial terga medially migrated and united. However, the entognathy of Diplura is constructed in a different manner, which is characterized by: 1) the mouth fold is formed by the fusion of extended terga of intercalary and three gnathal segments and labial coxa, 2) the postgnathal region of entognathy is derived from labial subcoxae which medially migrate and unite into a form of postmentum, and 3) the rotation of labial appendages is involved in the entognathy formation. The homology of entognathy is well supported between Protura and Collembola, hence the monophyly of Ellipura may be supported. While the manner of entognathy formation in Diplura is revealed to be much different from in Protura and Collembola either, hence the homology of entognathy between Protura-Collembola and Diplura is not always verified, and the monophyly of Entognatha could not be substantiated.

Keywords:*Comparative embryology, Phylogeny, Entognatha, Protura, Collembola, Diplura*

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Systematics, Phylogeny and Zoogeography

Unravelling Collembola phylogeny

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Among the three entognathan classes of hexapods (Protura Diplura and Collembola), springtails are arguably the most important. Their relationships have been discussed since the early 20^th century and despite recent attempts to solve their phylogeny, both from morphological and molecular data analyses, relationships among the main lineages and the very position of Collembola within Hexapoda are still largely open to debate. Multiple molecular loci and morphology for a broad taxonomic sampling are analysed through Direct Optimization to address the placement of Collembola, the four orders Neelipleona, Symphypleona, Entomobryomorpha and Poduromorpha, along with the familial relationships within the orders.

Keywords:*phylogeny, Hexapoda, Collembola*

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The molecular evolution of basal hexapods

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We have studied the phylogenetic positions of basal hexapods (Protura, Collembola and Diplura) by molecular approach for more than ten years. 18S, 28S rRNA genes, mitochondrial genomes and some nuclear protein-coding genes of the representative species from basal hexapods were sequenced and analyzed together with those of other arthropods. Our studies suggested it's hard to get a consistent tree and robust supporting values for the phylogenetic position of Protura, Collembola and Diplura by analyzing different molecular markers. Now, we are working their transcriptome data, and hope to figure out the most appropriate molecule markers for reconstructing the phylogeny of basal hexapods in Arthropoda.

Keywords:*Basal Hexapods, Molecular phylogeny*

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Phylogenetic analysis based on three nuclear DNA-encoded protein sequences confirms the monophyly of Hexapoda, but does not support the monophyly of Entognatha

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Molecular phylogenetic analyses have revealed that Hexapoda and Crustacea form a common clade (the Pancrustacea), which is now widely accepted among zoologists; however, the relationships between crustaceans and hexapods, and that among the basal lineages (Protura, Collembola, Diplura, and Ectognatha) of Hexapoda are still open questions. In this study we performed phylogenetic analyses using three nuclear genes encoding the catalytic subunit of DNA polymerase delta and the largest and second largest subunits of RNA polymerase II from 64 species of arthropods including all the hexapod orders. The amino acid sequences of these genes were subjected to phylogenetic inference based on the maximum likelihood and Bayesian methods. The resulting trees showed that all hexapods are clustered into a common clade with branchiopods being its sister lineage, while Crustacea is paraphyletic. Within Hexapoda, the monophyly of Entognatha (Protura, Collembola and Diplura), Ellipura (Protura + Collembola) and Nonoculata (Protura + Diplura) were not supported, and all resulting trees consistently suggested that Protura is the sister lineage to all other hexapods including collembolans and diplurans. Our results provide molecular evidence to support the possibility that Protura is the most basal lineage of hexapods, which is suggested from comparative embryology.

Keywords:*Hexapoda, Entognath, Molecular phylogeny, Nuclear gene sequence*

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Basal hexapod relationships based on phylogenomic data: Progress, challenges and pitfalls

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Phylogenetic relationships among apterygote hexapod lineages (Protura, Diplura, Collembola, Archaeognatha and Zygentoma) are still a matter of debate. In the last decades, several contradicting hypotheses, for example, "Ellipura" (Protura + Collembola) vs. "Nonoculata" (Protura+ Diplura) or the monophyly vs. a para- or polyphyly of hexapods, have been supported from either morphological, developmental and molecular studies. The inclusion of all primarily wingless orders is crucial to reliably resolve deep hexapod and euarthropod relationships, but the molecular data have been very sparse for these taxa. We provide new EST data (Expressed Sequence Tags) obtained from next generation sequencing techniques (NSG) covering all primary wingless hexapod orders. Within this project in which we use phylogenomic molecular sequence data, we carefully assessed data quality (e.g., orthology prediction, alignment masking, selection of genes with advanced selection heuristics). Our results corroborate the monophyly of Nonoculata, but show the need for research on the fate of non-random signal in phylogenomic datasets.

Keywords:*Phylogenomics, Hexapoda*

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Systematics, Phylogeny and Zoogeography

Can we ever hope for a robust backbone tree of hexapods?

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Phylogenetic relationships among apterygote hexapod lineages (Protura, Diplura, Collembola, Archaeognatha and Zygentoma) are still a matter of debate. In the last decades, several contradicting hypotheses, for example, "Ellipura" (Protura + Collembola) vs. "Nonoculata" (Protura+ Diplura) or the monophyly vs. a para- or polyphyly of hexapods, have been supported from either morphological, developmental and molecular studies. The inclusion of all primarily wingless orders is crucial to reliably resolve deep hexapod and euarthropod relationships, but the molecular data have been very sparse for these taxa. We provide new EST data (Expressed Sequence Tags) obtained from next generation sequencing techniques (NSG) covering all primary wingless hexapod orders. Within this project in which we use phylogenomic molecular sequence data, we carefully assessed data quality (e.g., orthology prediction, alignment masking, selection of genes with advanced selection heuristics). Our results corroborate the monophyly of Nonoculata, but show the need for research on the fate of non-random signal in phylogenomic datasets.

Keywords:*basal hexapods, origin of hexapods*

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Early splits of Pterygota: Inputs and shortcomings of the fossil record

Olivier Bethoux
France

With few mega-diverse sub-groups winged insects (Pterygota) compose the most diverse group of organisms on Earth. Multiple and comparatively recent evolutionary events are responsible for this diversity, but the acquisition of flight has been widely perceived as a trigger of a major and early radiation in the group. However the fossil record provides no conclusive evidence for such a radiation, or, more accurately, cannot be used to test this hypothesis. The earliest well-sampled faunas including Pterygota are early Late Carboniferous in age (~320 Myr ago). At this time Pterygota are represented by varied and distantly related groups, some of which already possessed refined flight capabilities, secondarily used wings as protective devices in association with cursorial locomotory behavior, and acquired specialized developmental pathways, all aspects incompatible with a near-contemporary flight-related radiation, and better suggestive of more ancient evolutionary history. Therefore it is assumed that pterygoty was acquired during the Early Carboniferous (at least), during which appropriate outcrops are rare, and, up to now, never yielded appropriate material. One of the collateral effects of this issue is that stem-relatives of the most major insect lineages (viz. palaeopterans, polyneopterans, Paraneoptera, and Holometabola) are unknown, although several stem-relatives at the ordinal level are well documented. Until productive outcrops are discovered, the 'Pterygota radiation' must be considered as speculative.

Keywords:*Fossil record, Pennsyvlanian, Mississipian, stem-groups*

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Resolving the phylogeny of non-endopterygote Hexapoda

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The origin and higher phylogeny of Hexapoda has been a topic of intense debate for decades, in particular the interordinal relationships. Relatively recent molecular data of Arthropoda support a sistergroup relationship between Xenocarida (Remipedia + Cephalocarida) and Hexapoda, and the underlying data and analyses are here critically discussed. Further, we present unpublished data from up to 27 nuclear, protein-coding genes (23kbp) for 58 taxa, representing most orders of non-endopterygote Hexapoda and expanding the arthropod data set to a total of up to 64 genes (42kbp) for 77 hexapods and 61 outgroup taxa. Maximum likelihood and Bayesian analyses of non-synonymous nucleotide substitutions robustly resolve most interordinal relationships. The resulting topology is congruent with those higher taxa that were well supported in previous studies, but provides additionally supported hypotheses for previously unresolved groupings. Particular attention is paid to the placement of Odonata and Ephemeroptera relative to Neoptera, of the 'living fossil' Tricholepidion gertschi to other Zygentoma, and to the phylogeny of Polyneoptera.

Keywords:*molecular phylogeny Hexapoda Polyneoptera*

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S116F01

Systematics, Phylogeny and Zoogeography

Phylogenetic relationships among superfamilies of Hymenoptera

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The results of a large-scale analysis of the Hymenoptera, published in 2011 in Cladistics, are summarized. Representatives of all extant superfamilies, were scored for 392 morphological characters, and sequence data for four loci (18S, 28S, COI and EF-1a). Including three outgroup taxa, 111 terminals were analyzed. Well supported relationships include: Xyeloidea is monophyletic, Cephoidea is the sister group of Siricoidea + [Xiphydrioidea + (Orussoidea + Apocrita)]; Anaxyelidae is included in the Siricoidea, and together they are the sister group of Xiphydrioidea + (Orussoidea + Apocrita); Orussoidea is the sister group of Apocrita, Apocrita is monophyletic; Evanioidea is monophyletic; Aculeata is the sister group of Evanioidea; Proctotrupomorpha is monophyletic; Ichneumonoidea is the sister group of Proctotrupomorpha; Platygastroidea is sister group to Cynipoidea, and together they are sister group to the remaining Proctotrupomorpha; Proctotrupoidea s. str. is monophyletic; Mymarommatoidea is the sister group of Chalcidoidea; Mymarommatoidea + Chalcidoidea + Diaprioidea is monophyletic. Weakly supported relationships include: Stephanoidea is the sister group of the remaining Apocrita; Diaprioidea is monophyletic; Ceraphronoidea is the sister group of Megalyroidea, which together form the sister group of [Trigonaloidea (Aculeata + Evanioidea)]. Aside from paraphyly of Vespoidea within Aculeata, an expected result, all currently recognized superfamilies are supported as monophyletic. The diapriid subfamily Ismarinae is raised to family status, Ismaridae stat. nov. The support for these relationships will be discussed and areas of uncertainty will be highlighted

Keywords:*Systematics, Cladistics*

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Phylogeny of the Chalcidoidea: Latest results and implications

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Chalcidoidea represent one of the greatest radiations of insects. More than 500,000 species have been estimated although only about 23,000 species have been described. The morphological diversity of the group is extreme, and there are many issues with classification of the approximately 85 subfamiles and 19 families of insects. Results are presented for a molecular analysis of 720 taxa based on an analysis of 18S and 28S gene regions, and a combined morphological analysis of 300 taxa. Morphology was scored for 232 characters using an online character coding system and a collaborative network of researchers. The combined results provide the first robust phylogeny of the Chalcidoidea and allow for a better approach to the understanding of various attributes including the evolution of heteronomy, photoplay, sternorryhncan parasitism, planidial larvae and other attributes.

Keywords:*Chalcidoidea, phylogeny, combined*

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Phylogeny of the Aculeata (Hymenoptera): Results of the Hymatol Taxonomic Working Group

Denis John Brothers[1], James M. Carpenter[2], Susanne Schulmeister[3], Donat Agosti[4], Celso O. Azevedo[5], Craig M. Brabant[6], Jacques Dubois[7], Lynn S. Kimsey[8], Volker Lohrmann[9], Michael Ohl[10], Laurence Packer[11]

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The first phylogenetic analysis based on combined morphological and molecular data for all families of Aculeata is presented. The morphological characters are modified from those used in previous studies by the authors, and are scored for more than 80 species terminals; they consist of 232 variables. The molecular data are from four genes (16S, 18S, 28S and COI), and comprise more than 3400 sites. Cladistic analysis using ichneumonoid outgroups resolves Chrysidoidea as monophyletic and the sister group of Apoidea and Vespoidea (that is, Aculeata s. str. is supported). Apoidea and Vespoidea are each monophyletic, and most families with multiple representatives are supported as monophyletic. The detailed results will be presented.

Keywords:*Chrysidoidea, Apoidea, Vespoidea, morphology, genes*

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Evolution of the hymenopteran ovipositor system: Implications for host exploitation

Donald L. J. Quicke
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Members of the order Hymenoptera tend to display considerable care in the placement of their eggs, be they phytophagous, parasitoids or predatory aculeates, and nearly always, the process of oviposition is accompanied by the deposition of various venoms and marking chemicals. This is achieved using the ovipositor and associated glands, which while being derived from the same basic structures throughout the order, display an enormous range of variation. The diversity of the ovipositor has enabled wasps to exploit a wide range of hosts, from eggs to adult insects, either exposed or deeply concealed. In this presentation I shall describe some of he many adaptive trends in hymenopteran ovipositors and show how they have both facilitated diversification and, in some cases, posed limitations on biological transitions.

Keywords:*oviposition, venom gland, eggs, endoparasitoid, parasitoid*

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S116F05

Systematics, Phylogeny and Zoogeography

Evolution of endoparasitism-strategies for dealing with the host milieu

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Parasitoid wasps have evolved a wide spectrum of developmental interactions with hosts. Here I synthesize and interpret results from the phylogenetic, ecological, physiological and molecular literature to identify factors that have influenced the evolution of endoparasitism. I first summarize major developmental strategies and assess the inventory of physiological and molecular traits that endoparasitoids use to successfully exploit hosts. I then discuss how certain traits have independently evolved in different lineages in likely response to similar host environments. Finally, I use a few specific examples to illustrate how phylogenetic, developmental and comparative genomic data can be used together to understand how some highly derived forms of endoparasitic development potentially evolved.

Keywords:*parasitoid wasp development embryogenesis*

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Host switching as biological transitions among parasitoids - exploiting spiders as a resource

Andrew D. Austin
The University of Adelaide, Australia

Spiders, their juvenile stages and eggs are utilised as prey/hosts by numerous insect groups, but particularly the Hymenoptera. Wasps attack spiders and their eggs as ecto- and endoparasitoids, or collect juvenile stages or adults as prey for their developing larvae, usually paralysing the spider and placing it within a nest or cell. Clearly, exploitation of spiders has evolved multiple times across the order, having been recorded by from four apocritan superfamilies and at least eight families. Of these the most diverse species radiations are found within the Platygastridae s.l. (as egg endoparasitoids) and Pompilidae (as juvenile/adult ectoparasitoids). With the recent advent of more robust phylogenies for hymenopteran families and genera it is possible to more accurately track the evolutionary transitions from utilising insect hosts to exploiting the various life history stages of spiders. Evident from this analysis is that there have been multiple independent switches to spider hosts/prey in all but one family, the Pompilidae, where exploiting spiders is an obligate family level trait. Further, where the biology of sister lineages is known two traits are implicated in the transition to utilising spiders, 1) the recognition of silk as a host-associated substrate (e.g. within the Platygastridae and Ichneumonidae) and 2) host searching within a specific habitat where several potential host/prey groups are found (e.g. Sphecidae). Possibly one of the most intriguing switches is found within the Pompilidae, where the ground-plan biology appears to be predation on spider eggs with a single switch to utilising juveniles/adults as prey for their larvae.

Keywords:*Hymenoptera, phylogeny, evolution, spiders, parasitoids,*

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S116F07

Systematics, Phylogeny and Zoogeography

Evolution of sex determination and endosymbionts in the Hymenoptera

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Sex determining mechanisms are bewildering diverse and turn over rapidly in evolution. Sex determination in insects occurs through a cascade of genetic switches with conserved genes at the bottom but strong diversification in the primary signal genes. I will discuss the genetic regulation and phylogenetic pattern of sex determination in the insect order Hymenoptera. All hymenopterans have haplodiploid reproduction (haploid males from unfertilised eggs, diploid females from fertilised eggs), but with different underlying genetic pathways of sex determination. Single locus complementary sex determination (slCSD) is known from >60 species in different families including Apoidea, Vespoidea and Ichneumonoidea. Within the family Braconidae slCSD and multi-locus CSD occurs in closely related species. Maternal effect genomic imprinting sex determination is only known from the chalcidoid Nasonia. Endosymbionts that alter their host reproduction and sex allocation occur widespread among Hymenoptera. They can induce cytoplasmic incompatibility, parthenogenesis, son killing and feminization. Their phylogenetic distribution is at least partially determined by the mode of sex determination. Recent evidence suggest that endosymbionts may directly manipulate the genetic cascade of sex determination.

Keywords:*endosymbionts, hymenoptera, sex determination*

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Defensive mutualisms and diffuse host-symbiont co-evolution in Hymenoptera

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In all stages of their life cycle, insects are threatened by predators, parasites, and pathogens. The lifestyles and feeding ecologies of some hymenopteran taxa render them especially vulnerable to pathogen infestation. Specifically, the development in subterranean brood cells, mass provisioning of resources for the offspring, and the living together of social insects in large communities can enhance the risk of pathogen infestation and/or the spread of disease among conspecifics. In addition to the host's own defenses, some Hymenoptera have evolved symbiotic associations with protective microorganisms. Here, we will give an overview on such defensive mutualisms in solitary and social Hymenoptera and discuss their evolutionary histories based primarily on the two best-studied model systems: the symbioses of antibiotic-producing Actinobacteria with leaf-cutter ants and solitary digger wasps, respectively. Pseudonocardia and Streptomyces bacteria associated with leaf-cutter ants protect the host's nutritional resources by inhibiting the growth of a parasitic fungus that can otherwise infect and overgrow the ants' cultivar fungus. Similarly, in digger wasps of the tribe Philanthini, individual Streptomyces strains produce a complex cocktail of at least nine different antibiotics on the wasps' cocoons, which provide protection against a wide range of opportunistic fungal pathogens. Phylogenetic analyses of both symbiotic associations reveal a complex pattern of diffuse host-symbiont co-evolution, with repeated uptake of novel symbionts from the environment (leaf-cutter ants) or horizontal transfer of symbionts among host species (digger wasps). Evidently, defensive symbiotic interactions can remain stable over long evolutionary timescales in the absence of strict vertical symbiont transmission and host-symbiont co-diversification.

Keywords:*Symbiosis, mutualism, leaf-cutter ants, beewolves, co-evolution.*

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Systematics, Phylogeny and Zoogeography

Tracing the rise of ants

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The evolution of the ants is increasingly well-understood due to recent analyses of phylogeny, divergence times and diversification rates. Yet leading hypotheses regarding the ancestral habitat of ants conflict with new findings that early ant lineages are cryptic and subterranean. Where the ants evolved, in respect to habitat, and how habitat shifts took place over time have not been formally tested. Here we reconstruct the habitat transitions of ants (Hymenoptera: Formicidae) through time, focusing on where they nest and forage (in the canopy, litter, or soil). We show that in contrast to the current consensus based on verbal arguments that ants evolved in tropical leaf litter, the ancestral stratum of all ants appears to be the soil with subsequent movement up into the litter and, in some cases, into the canopy. Given the global importance of ants, because of their diversity, ecological influence and status as the most successful eusocial lineage on earth, understanding the early evolution of this lineage has profound implications for understanding the factors that made this group so successful today.

Keywords:*ancestral state reconstruction, ecology, evolution, Formicidae, habitat, phylogeny*

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Uncovering the evolutionary history of a unique family of ant parasitoids (Eucharitidae: Hymenoptera)

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Eucharitidae (Hymenoptera) is unique in that it is the sole insect family comprised of only ant parasitoids. Eucharitid wasps are found worldwide, composed of 53 genera and potentially 700+ species. They are known to attack the immature stages of five subfamilies of ants, and have been found to be prevalent parasitoids among some important ant groups. Morphology, molecules, and shared life history traits unite the Eucharitidae as monophyletic. We present a fossil-calibrated molecular phylogeny (18S, 28S-D2-D5, COI, and COII) based on a dataset of over 350 taxa from the Eucharitidae and its sister group, the Perilampidae. Patterns of host ant use will be examined in consideration of the most recent eucharitid phylogeny and the updated formicid phylogenies. Statistical analyses include estimating diversification rate shifts within eucharitids, the ancestral state reconstruction of ant hosts, and cophylogeny reconstruction of the host-parasitoid phylogenies. We introduce a revised hypothesis of the correlation between the phylogenetic relationships of parasitoid and host. Estimates from molecular divergence dating studies suggest an origin of Eucharitidae at roughly 65-75 mya, while major ant lineages diverged much earlier, well over 100 mya. Eucharitids display phylogenetic conservatism of ant hosts at the subfamily level, but the current pattern of lower-level host associations in Eucharitidae is best explained by host-switching and colonization throughout much of its evolutionary history. There may well be coevolution between the two hymenopteran groups due to the intimacy of the association.

Keywords:*Eucharitidae, parasitoid, ant, cophylogeny*

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Morphology and phylogeny of the enigmatic wasp genus Cales (Chalcidoidea: Calesinae)

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The hymenopteran superfamily Chalcidoidea currently contains about 22,000 described species in 19 families, with estimates of total species ranging from 100,000 to 500,000 worldwide. Despite the importance of many species as biological control agents, the species-level diversity and phylogenetic relationships within the group are poorly understood. The family Aphelinidae is among the chalcidoid groups most in need of systematic attention. These are small wasps ranging from 0.5 to 2.5 mm total body length that are mostly parasitoids of sternorrhynchus Hemiptera. This work will address species-level relationships within the unusual genus Cales, which has historically been associated with Aphelinidae, but is of questionable taxonomic position at this time. The phylogenetic placement of Cales within Chalcidoidea is uncertain, and is currently unplaced within Chalcidoidea. Molecular studies suggest that Cales is a distinct group unrelated to other Aphelinidae, though analyses have thus far failed to identify a well-supported sister-group relationship. There is currently one described species from the new world, Cales noacki, which has been introduced and established in citrus-growing regions of North America and Mediterranean countries, and has been relatively successful in reducing woolly whitefly populations. However, molecular analyses presented here suggest that many genetically distinct variants of C. noacki are present within its biological control and native ranges. These genetic variants appear to be morphologically indistinguishable, though detailed morphometric analyses are needed. Issues with taxonomic descriptions of cryptic species complexes are discussed.

Keywords:*cryptic species, biological control*

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Arthropod aloft: The origins of aerial behavior and flight

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Diverse insect taxa exhibit remarkable aerial capacities, including jumping, mid-air righting, parachuting, gliding, landing, controlled maneuvers, and flapping flight. The origin of flapping wings in hexapods greatly facilitated subsequent diversification, but both the paleobiological context and possible selective pressures for the evolution of wings remain contentious. Larvae of various arboreal hemimetabolous insects, as well as many adult canopy ants, demonstrate the capacity for directed aerial descent in the absence of wings. Aerial control in the ancestrally wingless archaeognathans suggests that flight behavior preceded the origins of wings in hexapods. In evolutionary terms, the use of winglets and partial wings to effect aerial righting and maneuvers could select for enhanced appendicular motions, and ultimately lead to powered flight. Flight behaviors that involve neither flapping nor wings are likely to be much more widespread than is currently recognized. Further characterization of the sensory and biomechanical mechanisms used by these aerially capable taxa can potentially assist in reconstruction of ancestral winged morphologies and facilitate our understanding of the origins of flight.

Keywords:*bristletail, flight, gliding, hexapod, wings*

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Multimodal sensory control of insect flight

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Insects use diverse sensory modalities including vision and mechanosensation to control flight in the rapid timescales. Sensory information transduced by various sensory organs is integrated within the brain which processes these diverse inputs and generates appropriate motor outputs to maintain stable flight. The chain of events from sensory perception to its processing and motor control must often happen within very short time scales, and hence presents a challenge to the insect nervous system. We have recently been exploring the mechanisms by which insects use multimodal sensory inputs to control their flight. Specifically, I will discuss two cases studies investigating the mechanosensory control of flight. In the first case study, I will describe how the antennae of moths (and other four-winged insects) provide mechanosensory cues that are critical for flight control. Here, I will describe the neural mechanisms that underlie the antennal control of flight, as well as the fine positioning of the antenna during flight. Furthermore, these inputs are strongly influenced by visual feedback. In the second case study, I will describe how the kinematics of wings and halteres of Dipteran insects remain in tight coordination in spite of the fact that their wing beat frequencies can often be on the order of a few hundred per second. Together, these studies shed some light on how insect morphologies and their sensory structures have evolved to meet the challenges of providing rapid sensory feedback during flight.

Keywords:*Mechanosensory, vision, insect flight*

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Dynamic flight stability of a bumblebee in forward flight

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The longitudinal dynamic flight stability of a bumblebee in forward flight is studied. The method of computational fluid dynamics is used to compute the aerodynamic derivatives and the techniques of eigenvalue and eigenvector analysis are employed for solving the equations of motion. The primary findings are following. The forward flight of the bumblebee is not dynamically stable due to the existence of one (or two) unstable or approximately neutrally stable natural modes of motion. At hovering to medium flight speed (flight speed U=0-3.5 m/s; advance ratio J=0-0.44), the flight is weakly unstable or approximately neutrally stable; at high speed (U=4.5 m/s; J=0.57), the flight becomes strongly unstable (initial disturbance double its value in only 3.5 wingbeats).

Keywords:*Bumblebee, dynamic stability, forward flight, Navier-Stokes simulation, natural modes of motion*

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Ecophysiology of migratory flight in Lepidoptera

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Juvenile hormone is an important determinant in the response of migratory insects to predictable or unpredictable deterioration of habitat quality. Low titers, produced in response to cues indicating a decline in habitat quality, stimulate migratory behaviour while under favourable conditions high JH titers initiate reproductive maturation. However, it is still not entirely clear if sexual maturation only occurs once migratory flight has terminated or whether it may occur during the migratory process. If, as suggested from recent results obtained with Mythimna separata, reproductively active individuals migrate, then the costs associated with continuous long distance flight on future reproductive output may differ between immature and sexually mature individuals. In this talk I will discuss adaptations that may reduce these costs and the importance of using individuals reared under field conditions, on natural diets, to address these questions experimentally.

Keywords:*Lepidoptera, Juvenile Hormone, Migratory flight, Reproductive success, Trade-offs*

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Reproductive benefits of seasonal insect migration to high latitudes

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Billions of insects migrate between winter and summer ranges to take advantage of seasonally-available breeding resources. To cover the distances required (hundreds of kilometers), many insects rely on wind assistance, and routinely ascend hundreds of meters above the ground to migrate in fast-moving airstreams. Given that wind speeds are typically three to five times faster that the insects' airspeeds, it was not clear what influence high-flying migrants could exert on their migration direction or whether substantial 'return' migrations to lower-latitude winter-breeding areas were possible. To answer this question, I have studied the flight behavior and migration patterns of large nocturnal moths (including Autographa gamma and Noctua pronuba) in Europe with specialized entomological radars. Radar observations demonstrated that an ability to select favorably-directed airstreams (i.e. northwards in spring and southwards in autumn) is widespread among high-flying migrant Lepidoptera, and thus the migrants gained considerable wind assistance for their seasonal migrations. Furthermore, moths preferentially fly at the altitude of the fastest winds, and partially compensated for wind drift away from their seasonally-preferred migration directions. Trajectory simulations show that these flight behaviors result in significant increases in mean nightly migration distance, and a high degree of success in reaching the next breeding region, while population monitoring indicated that high-latitude summer-breeding results in a fourfold population increase. The migration strategies employed by the study species explain how such small, short-lived and relatively slow-flying organisms are able to cover such great distances in seasonally-beneficial directions, and demonstrate that migration is highly adaptive in such cases.

Keywords:*Migration, Lepidoptera, Orientation, Radar Entomology*

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Orthopteran mass migrations

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Orthopterans such as swarming locusts and Mormon crickets provide some of the most spectacular examples of insect mass migration. Swarms can contain billions of individuals, and in the case of flying adult locusts, are capable of extended flights across continents and oceans. These insect mass movements have been noted by humans throughout history, in no small part due to their ecological and cultural impact as agricultural pests. However, our understanding of the proximate and ultimate mechanisms underlying swarm formation and movement has changed dramatically in a relatively short amount of time. I will provide an overview of these advances, highlighting recent findings across disciplines ranging from radiotelemetry and molecular population genetics to collective dynamics and nutritional ecology.

Keywords:*swarm, migration, locust, Mormon cricket, radiotelemetry, population genetics, collective movement, nutritional ecology*

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Orientation behaviour of nocturnal insect migrants observed with a monitoring radar

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Nocturnal insect migration over the extensive inland plain of southeastern Australia has been observed over several seasons with an Insect Monitoring Radar (IMR) located at Bourke, New South Wales. The species detected are primarily Australian plague locusts, Chortoicetes terminifera, and a variety of moths (mostly Noctuidae). Directed movements involving numerous individuals ? i.e. migrations ? are frequently detected between August (early spring) and April (mid-autumn). For these nights, the number of targets providing good-quality echo signals, i.e. signals from which all trajectory and target-character parameters can be retrieved, is in the low thousands. This allows examination of sample variances and of variations with height and time of night, and sometimes also comparison of the simultaneous behaviours of different migrant types. The radar observations show that a degree of common orientation is almost invariably present in the stable atmospheric conditions in which nocturnal insect migration typically occurs, but that the range of orientation angles is often broad. Mean orientations vary with both height and time, and these changes appear to be associated with changes in the movement directions of the insects, with these in turn being determined primarily by the wind. However, there is often an offset between the orientation and movement directions, and this offset seems to vary with the movement direction. With migrations occurring in all directions at this site (in the warmer months), and more than one target type quite often present, a careful analysis may eventually reveal the cues and processes producing the orientation phenomena observed there.

Keywords:*insect, migration, orientation*

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Orientation and layering behaviors of nocturnal migrant insects revealed with entomological radar in northern China

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The high altitude migrations of nocturnal migrant insects over both continent and sea have been observed with entomological radar and light traps in the past decade in northern China. The insects being observed include beet armyworm Spodoptera exigua, beet webworm Loxostege sticticalis, cotton bollworm Helicoverpa armigera, oriental armyworm Mythimna separata, dragonflies Pantala flavescens and ground beetles Pseudoophonus griseus. Orientation and layering were the two most attractive behaviors of these nocturnal migrants. Migrant insects tended to heading towards the northeast during spring migration, but southwest during autumn migration, with a general pattern of heading downwind. Some strong migrants could sometimes head across the wind or even upwind. Other less strong migrants, such as H. armigera, headed towards the southwest in varied downwind directions during autumn. The M. separata was used to study the possible mechanisms of orientation during migration with a tethered flight simulator. It could orient toward northeast in spring but southwest in autumn without the cues of winds and lights, which indicated M. separata might have used the geographic magnetic field as the cues for orientation. Layering was a complicated behavior and might be a integrated reaction to temperature, wind speed and wind direction through space (vertical profile) and time (season). A simple model basing on statistics has been built up to simulate the layering behavior. Apparently, more elaborated experiments and sophisticated statistics were desired to explore the mechanism of orientation and layering behaviors in nocturnal migrants.

Keywords:*Migration, Radar, Orientation, Layering*

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Radar entomology in Japan

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Many long-distance migratory insects come into Japan. Among them, rice planthoppers are important insect pests of rice. To know the timing and area of their immigrations in advance, a migration prediction method has been developed. A real-time prediction system using the method is now operational and the information is delivered to plant protection officers. Its simulation model employs the knowledge on the insect flight unveiled by an entomological radar in 1980s. To improve the prediction accuracy, it is necessary to approach real images of immigrants in flight over western Japan, such as their aerial density at altitudes and an unknown landing process. It is then to feed back the information to modification of the model. Currently we have two types of entomological radar: one is a Vertical Looking Radar-type in x-band, the other a 95 GHz FMCW-type millimetric radar. The former targets middle-sized moths, and is currently under construction. The latter targets small-sized insects, and was tested during a wheat harvesting season in early summer, 2011. Its observation was compared with the monitoring data by a net trap dangled under a balloon. Many target echoes were observed in daytime and at dusk on sunny days, but a small number at dawn and in early morning, and few in night time. The aerial density of echoes was estimated and found to be comparable to that of the net trap monitoring. The size of echoes estimated by a spherical water droplet approximation ranged from 1 to 0.2 mm.

Keywords:*entomological radar, migration simulation, FMCW millimetric radar*

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The role of FoxO in integrating insulin and ecdysone signaling during body size regulation

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Body size regulation, a conspicuous and fundamental process in any organism, is best understood in insects. In the fruit fly Drosophila melanogaster, larvae regulate their final body size by controlling both growth rate, through insulin/insulin-like growth factor signaling (IIS) and target of rapamycin signaling, and regulating the duration of the growth period, via the steroid hormone ecdysone. Recent studies have shown that these two processes interact; IIS stimulates ecdysone synthesis from the prothoracic gland by up-regulating ecdysone biosynthesis genes. How this occurs is still unclear. In mammalian and nematode cells, the IIS modulates steroid hormone signaling through the interaction between a downstream component of the IIS, forkhead box type O (FoxO), and nuclear hormone receptor superfamily members. Given this, we have found that a heterodimeric partner of Ecdysone Receptor, Ultraspiracle (Usp), binds to FoxO. Furthermore, we found that overexpression of FoxO RNAi in the prothoracic gland (PG) induces premature patterning of the imaginal discs in early third instar larvae fed on sucrose alone, suggesting ecdysone synthesis occurs prematurely. This FoxO/Usp association occurs independently of 20-hydroxyecdysone, suggesting that the complex may potentially regulate ecdysone signaling in a nutrient-dependent, but ecdysone-independent manner. To explore the function of FoxO/Usp complex, we have created two different types of transgenes that inhibit the formation of FoxO/Usp complexes without disrupting independent transcriptional activity of FoxO. We will discuss about the function of the FoxO/Usp complex on growth period determination.

Keywords:*Drosophila, insulin signaling, ecdysone, body size control, Foxo, Usp*

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The function of Rab4b in insect growth and development

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The insulin and 20-hydroxyecdysone (20E) pathways coordinately regulate insect growth and metamorphosis. However, the molecular mechanism of the interaction of these two pathways in regulating insect development is not well understood. In the present study, we found that a small GTPase Rab4b from a lepidopteran insect Helicoverpa armigera participates in signal transduction in the two pathways. The results show that RNA interference of Rab4b in larvae results in larval death, abnormal metamorphic transition or small pupae, and the decrease of glycogen levels. The molecular mechanisms were demonstrated that knockdown of Rab4b in the larvae suppresses the transcription of glycogen synthase (GS), as well as the metamorphic-initiating factor (Br) and hormone receptor 3 (HR3), but increases the transcription of Forkhead box class O (FOXO). Further studies in the cell line confirm that Rab4b is necessary for gene transcription in the insulin and 20E pathways. Rab4b locates in the cytoplasm and keeps FOXO in the cytoplasm in the insulin pathway, but travels toward the cell membrane upon 20E induction without affecting the FOXO location. The transcription of Rab4b could be upregulated by insulin injection or glucose feeding to the larvae, but not by 20E or juvenile hormone analogy methoprene. Our data suggest that Rab4b plays roles in insect growth and metamorphosis by regulating glycogen synthesis gene transcription in the insulin and 20E pathways. This work was supported by the grants from the National Natural Science Foundation of China (No. 30970404) and National Basic Research Program of China (973 Program, 2012CB114101).

Keywords:*Rab4b, insulin pathway, 20-hydroxyecdysone pathway, FOXO*

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Balancing crosstalk between steroid-induced autophagy and apoptosis in *Drosophila* remodeling fat body

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Apoptosis and autophagy, two types of programmed cell death (PCD), play critical roles during development in animals. Common stimuli can induce combined autophagy and apoptosis; in many instances, the two PCD processes antagonize each other and develop in a mutually exclusively manner. In this study, we discovered that during the larval-pupal transition in Drosophila, a balancing crosstalk occurs between autophagy and apoptosis in the remodeling fat body: the inhibition of autophagy induces apoptosis and the inhibition of apoptosis induces autophagy. Both PCD processes are induced by the steroid hormone, 20-hydroxyecdysone (20E), via its nuclear receptor, EcR-USP. E93, a 20E primary-response gene encoding a HTH transcription factor, plays a predominant role in coordinating 20E-induced PCD, mainly because E93 upregulates the genes responsible for both autophagy and apoptosis and inhibits the PI3K-TORC1 signaling. The Drosophila remodeling fat body provides an in vivo model for understanding the molecular mechanism of the balancing crosstalk between autophagy and apoptosis, which are induced by 20E and coordinately mediated by E93.

Keywords:*Autophagy, Apoptosis, 20-hydroxyecdysone, EcR-USP, E93, Drosophila melanogaster*

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Mechanisms and evolution of nutrient-dependent horn allometry in beetles

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Our research focuses on the genetic mechanisms of conditional-expression and trait exaggeration in weapons of sexual selection. We study these mechanisms of weapon growth in three different lineages of scarab beetles, encompassing the three principle origins of exaggerated weapons of sexual selection in this superfamily: horns of dung beetles (Scarabaeinae) and rhinoceros beetles (Dynastinae), and mandibles of stag beetles (Lucanidae). In the process, we have developed genomic tools and resources for all three lineages. By examining how traits grow to extreme proportions, we have begun to elucidate the underlying physiological and genetic pathways regulating the rate of trait growth, and coupling growth with environmental factors such as nutrition and with individual body condition. We have focused on insulin and insulin-like growth factors because they have been shown to be essential for regulating tissue growth and body size and because their role has been largely conserved among metazoans. We predicted that increased cellular sensitivity to the insulin/IGF pathway within specific trait primordia might be a mechanism for the evolution of disproportionate, or exaggerated, growth in the showy ornaments and weapons of sexual selection. Here, we present results from our studies of the insulin receptor in the rhinoceros beetle and stag beetle which support the hypothesis that the insulin/IGF pathway contributes to exaggerated growth of animal weapons.

Keywords:*beetle, horn, mandible, exaggerated growth, insulin signaling*

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The regulation of organ allometry in *Drosophila*

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Morphological diversity between species is dominated by variation in body proportion; that is the size of individual organs relative to the size of the body as a whole. Nevertheless, we have a very poor understanding of how body proportion is regulated developmentally and how this regulation evolves. Using Drosophila as a model organism, we identify the developmental mechanisms that control how organ size scales with body size, that is organ allometry. These mechanisms control morphological scaling and body proportion by regulating the extent to which body and organ size respond to environmental, and likely genetic, variation. We use mathematical modeling to explore how evolution may act on these mechanisms to alter scaling relationships . Finally, we show how artificial selection can target these mechanisms and alter the allometric relationship between organ and body size in Drosophila.

Keywords:*phenotypic plasicity, insulin signaling, nutrition, mathematical modeling, artificial selection*

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Is internal hypoxia a signal for the transition from growth to molting in insect larvae?

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Body size profoundly affects many aspects of animal biology, yet the mechanisms responsible for determining the size of animals remains one of the fundamental unsolved problems of developmental biology. Insects and other arthropods add substantial mass and oxygen need within each larval instar, while many aspects of the tracheal respiratory system remain fixed in size. Thus sensing of reduced internal PO2 could be one mechanism that leads to cessation of larval growth and initiation of the molting cascade, which occurs when the larva attains a critical weight. Metabolic rates of third instar Manduca sexta and Drosophila melanogaster larvae increase linearly with mass before the critical weight, and remain constant as mass increases after the critical weight, supporting the concept of oxygen-limitation later in the instar. Rearing in hypoxia causes a dose-dependent decrease in adult size in mostinsects, consistent with the hypothesis that oxygen-limitation in the final instar determines the size at which metamorphosis is initiated, and therefore the final body size.Hypoxic exposure during the third larval instar reduces feeding rate, with a consequent effect on growth rate. Hypoxic rearing shifts the critical weight to a smaller size, consistent with hypoxic-regulation of development. Despite the smaller critical weight, hypoxia delays pupariation. Late-third instar larvae grown in normoxiashow elevated levels of hypoxia-inducible factor (HIF) signaling, suggesting that HIF-signaling of reduced PO2 levels could be a component of the signaling pathway that reduces larval feeding, growth and critical weight, and promotes transition to molting and adult size determination.

Keywords:*internal hypoxia*

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S301M01

Insect Immunology, Physiology and Neurobiology

Hemolymph protease cascades in immune responses of *Manduca sexta*

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Extracellular serine protease cascades mediate rapid responses to infection and wounding in animals. Such cascade pathways offer mechanisms for rapid, local amplification of a small initial signal, with potential for regulation at multiple levels. The proteases circulate as inactive zymogens and become sequentially activated upon recognition of aberrant tissues or microbial polysaccharides. After accomplishing their functions, the active enzymes are often inactivated by serine protease inhibitors. It is becoming apparent that protease cascade pathways in blood are often connected, creating protease "webs," and this is likely to be true in Manduca sexta and other insect species. Activation of serine proteases in hemolymph is a component of several types of lepidopteran immune responses, including melanotic encapsulation, activation of antimicrobial peptide synthesis, and modulation of hemocyte function. Many insect hemolymph serine proteases are composed of a carboxyl-terminal protease domain and an amino-terminal clip domain. Clip domains are 35-55 amino acid residue sequences that may mediate interactions of members of protease cascade pathways. We have cloned cDNAs for more than 20 clip domain proteases expressed in fat body or hemocytes of Manduca sexta and are investigating their functions in immune responses and their regulation by members of the serpin family of protease inhibitors. Hemolymph protease pathways leading to activation of prophenoloxidase and activation of the cytokine, spatzle, will be discussed, as well as regulation of these pathways by inhibitory serpins. Initial observations on hemolymph proteases and serpins from the Manduca sexta genome sequence will be provided. (supported by NIH grants GM41247 and GM58634)

Keywords:*hemolymph, clip domain, protease, serpin, innate immunity, pattern recognition*

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S301M02

Insect Immunology, Physiology and Neurobiology

Identification of bacterial genes expressed upon infection to control pathogenicity in *Drosophila*

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Upon infection bacteria appear to adapt to the host environment by altering the pattern of gene expression. Most probably, host substances including those that constitute the host immune system induce this change in invading bacteria. To elucidate the mechanism of bacterial gene regulation and characterize newly expressed genes should deepen our understanding of the behavior of bacteria in host organisms as well as the state of infections diseases. To identify bacterial genes that are induced upon infection, we examined the level of expression of Escherichia coli genes coding for proteins that control gene expression, i.e. sigma subunits of RNA polymerase and proteins involved in the two-component sensor-transducer response system, in Drosophila melanogaster that had been abdominally injected with E. coli. We then determined survival ratios of adult flies after abdominal infection with E. coli that harbor mutations in the genes identified. We found that many E. coli mutants showed altered levels of pathogenicity compared to their parental strain, indicating that sigma subunits and two-component systems are involved in the control of pathogenicity of E. coli to Drosophila. These proteins are presumed to induce transcription of a set of genes leading to the production of pathogenicity-related proteins. This is most likely a bacterial strategy for adaptation to the host environment to achieve persistency in host organisms coping with immune reactions. It is probable that bacteria change the pattern of gene expression when exposed to host substances, hemolymph proteins in particular, but precise mechanisms await further investigation.

Keywords:*Drosophila, bacterial infection, gene expression, sigma factor, two-component system*

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Immune proteins in hemolymph of *Galleria mellonella*

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Galleria mellonella (Greater wax moth) might be one of appropriate models in that it arms itself with a variety of hemolymph proteins to kill and eliminate invading microorganisms. Aside from prophenoloxidase (PPO) system, there are at least five types of immune proteins in the hemolymph of G. mellonella. As is usual in most of insects, G. mellonella has a cecropin and a lysozyme as inducible antimicrobial proteins. Apolipophorin-III that is known to involve in the lipid metabolism might contribute to humoral and cellular immune reactions triggering in the hemolymph upon microbial invasion. In addition, a small protein (9.1 kDa), named GmCP8, was demonstrated to play a role as a multiligand receptor to detect diverse polysaccharides (LPS, LTA and beta-1,3-glucan) present in the microbial cell wall, thereby contributing substantially to phagocytosis of microorganisms by hemocytes. As the fifth immune protein found in the hemolymph of G. mellonella, Gm protein-24 was confirmed to be antimicrobial protein capable of killing gram-positive bacteria but not gram-negative bacteria. The protein has strong affinity to teichoic acid that is the outermost component of cell wall of gram-positive bacteria, showing anti-staphylococci activity. Intriguingly, it was shown that Gm protein-24 exerted its anti-staphylococci activity in a mode of action distinctly different from conventional antimicrobial peptide such as cecropin. Overall, a better understanding of immune proteins occurring in the hemolymph of G. mellonella might provide us with critical insight into the insect defense mechanisms.

Keywords:*G. mellonella hemolymph protein*

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S301M04

Insect Immunology, Physiology and Neurobiology

Antiviral and "specific" immunity in a crustacean

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The circulating blood cells (hemocytes) are crucial for protecting invertebrates against invading microorganisms, and they are directly involved in recognition, phagocytosis, melanization and cytotoxic reactions. Many microorganisms trigger hemocyte synthesis and release from the HPT, and this may be one way for the host to respond to an infection. Therefore we have studied hematopoiesis intensely and have now in detail deciphered the molecular mechanisms in hematopoietic stem cell proliferation and differentiation. Recently we have cloned Dscam proteins from P. leniusculus. The Down syndrome cell adhesion molecule (Dscam) is a member of the immunoglobulin super family of proteins. Dscams are large proteins containing ten Ig domains and six fibronectin type III domains, and this structure is conserved across animal phyla. There are two Dscam genes in humans (DSCAM), and they have an essential role in neural wiring, in both vertebrate and insects. An intriguing difference in Dscam gene structure is the exon duplication in arthropods and which give rise to an array of alternative spliced isoforms in insects and crustaceans. Alternative splicing of individual exon sequences allows for expression of several thousands of isoforms . We have now shown that hemocytes that express different spliced isoforms are induced by specific challenge, and moreover that these specific forms are important in clearing bacteria. Finally we are also studying the antiviral response in crayfish and shrimp and found a new mechanism by which the White spot syndrome virus is able to replicate in the hemocytes.

Keywords:*hematopoiesis,Dscam, Virus replication*

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S301M05

Insect Immunology, Physiology and Neurobiology

A link coordinating humoral and cellular responses in *Drosophila* immunity

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The fruit fly Drosophila melanogaster is an excellent model organism to decipher the basic principles of innate immune responses consisting with humoral and cellular responses. One of the humoral responses is induction of antimicrobial peptides (AMPs), that is controlled by two distinct innate immune signaling pathways, the Toll and immune deficiency (imd) pathways. The Toll pathway is mediated by Toll receptor and dMyD88 adaptor protein, the imd pathway is mediated by Imd adaptor protein. The cellular responses are mainly caused by blood cells called hemocytes. To identity genes capable of activating immune responses, we have established a genome wide gain-of-function genetic screen in Drosophila and identified peptidoglycan recognition protein -LE recognizing diaminopimeric acid-type peptidoglycans and activating imd-dependent AMP synthesis and autophagy (PNAS 2002, EMBO J. 2004, Nature Immunol. 2006, 2008). Here, using the genetic screen, we identified a receptor guanylyl cyclase (rGC). The rGC shows amino acid sequence similarity to mammalian receptor guanylyl cyclases producing cyclic GMP such as Atrial Natriuretic Peptide receptor. The rGC is involved in both Toll-independent/dMyD88-dependent humoral response and dMyD88-independent cellular response, that is crucial for host survival against Gram-positive bacterial infections but not against Gram-negative bacterial infections.

Keywords:*innate immunity cGMP receptor guanylyl cyclase*

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Hemolymph pattern recognition proteins involved in innate immune responses of *Tenebrio molitor*

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Innate immunity is a crucial host defense mechanism against microbial infection in all animals. The insect's Toll proteolytic signaling cascade is activated upon recognition of Gram-positive bacteria and fungi, resulting in the expression of antimicrobial peptides via NF-kappaB-like transcription factor. These innate immune responses are mediated by a serine protease cascade leading to the processing of pro-Spatzle, which generates the functional ligand of the Toll receptor. Recently, we analyzed the serine protease cascade regulating the Toll signaling pathway using larvae of the beetle, Tenebrio molitor. This large insect enabled us to collect large amount of hemolymph (the insect blood) allowing purification of serine proteases and pathogen recognition receptors. Our studies demonstrated that the recognition of bacterial Lys-type peptidoglycan (PG) by the PG recognition protein-SA (PGRP-SA)/Gram-negative binding protein 1 (GNBP1) complex activates pro-Spatzle via the sequential activation of three serine proteases: modular serine protease (MSP), Spatzle processing enzyme-activating enzyme (SAE) and Spatzle processing enzyme (SPE). Additionally, we provide biochemical evidences of how the Tenebrio PGRP-SA/GNBP 1 complex-mediated bacteria recognition signal is transferred to pro-Spatzle leading to the production of antimicrobial peptides. Injection of Lys-type PG into Tenebrio larvae induced production of two antimicrobial peptides, Tenecin 1 and Tenecin 2, which are also inducible by injection of the active form of processed Spatzle. Furthermore, we identified five different serpins that are involved in the regulation of Toll signaling cascade and melanin synthesis pathway. We also examined in vitro and in vivo biological functions of these serpins during Toll activation cascade.

Keywords:*innate immunity, pattern recognition proteins, Toll, pathogens, Tenebrio molitor*

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S302M01

Insect Immunology, Physiology and Neurobiology

Deploying the mosquito IMD pathway in the war against malaria

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A causative agent of human malaria, Plasmodium falciparum, is transmitted by Anopheles mosquitoes. The malaria parasite is under intensive attack from the mosquito's innate immune system during its sporogonic development. We have used genetic engineering to create immune-enhanced Anopheles stephensi mosquitoes through blood meal-inducible expression of a transgene encoding the IMD pathway-controlled NF-kB Rel2 transcription factor in the midgut and fat-body tissue. We have determined the anti-P. falciparum circuit of the IMD pathway and identified several pathway factors that could serve as tools for the development of genetically modified super-immune mosquitoes. Rel2 transgenic mosquitoes showed greater resistance to Plasmodium and microbial infection as a result of timely concerted tissue-specific immune attacks involving multiple effectors. The relatively weak impact of this genetic modification on mosquito fitness under laboratory conditions encourages further investigation of this approach for malaria control.

Keywords:*Anopheles, malaria, innate immunity, transgenesis*

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S302M02

Insect Immunology, Physiology and Neurobiology

Biological functions of insect cytokine growth-blocking peptide (GBP)

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The expression of antimicrobial peptide genes (AMPs) is induced by septic injury under the control of the Toll and immune deficiency (Imd) pathways in Drosophila melanogaster, and Spatzle is the known insect cytokine that stimulates one of these pathways, Toll. Here we demonstrated that growth-blocking peptide (GBP) is also a potent cytokine that activates AMP expression in insects under various stress conditions. Injection of silkworm Bombyx mori GBP enhanced transcription of AMPs in B. mori larvae. To extend this finding to the Drosophila immune system, we identified Drosophila GBP through isolation and sequencing of the bluebottle fly Lucilia cuprina GBP. Overexpression of GBP in Drosophila larvae elevated expression of AMPs such as Metchnikowin and Diptericin in all tested tissues, fat body, hemocytes, and integuments. GBP-induced AMP expression in Drosophila larvae did not require orthodox Toll and Imd pathway-related genes such as spatzle, dorsal, Dorsal-related immune factor, peptidoglycan-recognition protein-LC (PGRP-LC), PGRP-LE, and Relish, but imd itself and basket were essential, indicating that this signaling is activated by association with the adaptor protein Imd to enhance AMP expression through the Jun N-terminal kinase pathway. RNAi knockdown of GBP expression prevented the bacterial challenge-induced AMP expression, and enhanced susceptibility to the bacteria. The enhancement of AMP expression by noninfectious stresses such as subcutaneous damage and temperature fluctuation was apparent in control larvae but not in GBP-knockdown larvae. These results indicate that the Drosophila GBP signaling pathway mediates acute innate immune reactions after various stress stimuli, regardless of whether they are infectious or noninfectious.

Keywords:*Insect cytokine, Growth-blocking peptide (GBP), stress, innate immunity*

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Both biogenic monoamine and PSP activate hemocyte-spreading via eicosanoids

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Insect innate immunity is composed of humoral and hemocytic (or cellular) responses. Plasmatocyte spreading peptide (PSP), the first reported insect cytokine, is responsible for mediating the spreading and attachment of some sub-classes of plasmatocytes to nodules. Prostaglandins (PGs), one group of eicosanoids formed from arachidonic acid (AA), also mediate plasmatocyte spreading (PS), although the potential interactions between the PSP and PG signal transduction pathways have not been investigated. We tested our hypothesis that PSP acts via biosynthesis of eicosanoids, specifically PGs, in the beet armyworm, Spodoptera exigua. Here we report that PSP and PGE2 independently stimulated Ca++-dependent PS, that inhibitors of PG biosynthesis reversibly blocked PS, that dsRNA silencing the gene encoding proPSP blocked PS, which was rescued by PSP and by AA, that PSP-stimulated PS was reversibly impaired by inhibitors of PG biosynthesis, and the inhibitor-impaired spreading was rescued by AA. Taken together, these points strongly support our model showing that PSP acts via a plasmatocyte-surface receptor, which stimulates biosynthesis of the PGs responsible for mediating plasmatocytes spreading. In addition, biogenic monoamines, 5-hydroxytryptamine and octopamine, also activate hemocyte spreading behavior via eicosanoids. These studies suggest that eicosanoids are the end signaling molecules activating hemocyte behavior after cross-talks with other cytokines in response to pathogen infection.

Keywords:*Plasmatocytes spreading peptide, Prostaglandins, Plasmatocytes, Insect immunity, Biogenic monoamines*

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S302M04

Insect Immunology, Physiology and Neurobiology S302

Hindgut innate immunity and regulation of fecal microbiota through melanization in insects

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Many insects eat the green leaves of plants but excrete black feces in a yet-unknown mechanism. Insects cannot avoid ingesting pathogens with food that will be specifically detected by the midgut immune system. However, just as in mammals, many pathogens can still escape the insect midgut immune system and arrive in the hindgut, where they are excreted out with the feces. Here we show that the melanization of hindgut content induced by prophenoloxidase, a key enzyme that induces the production of melanin around invaders and at wound sites, is the last line of immunity to clear bacteria before feces excretion. We used the silkworm Bombyx mori as a model and found that prophenoloxidase produced by hindgut cells and is secreted into the hindgut contents. Several experiments were done to clearly demonstrate that the blackening of the insect feces was due to activated phenoloxidase, which served to regulate the number of bacteria in the hindgut. Our analysis of the silkworm hindgut prophenoloxidase disclose the natural secret why the insect feces is black and provides insight into the hindgut innate immunity that is still rather unclear in mammals.

Keywords:*hind gut, innate immunity, prophenoloxidase, fecal microbiota*

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S302M05

Insect Immunology, Physiology and Neurobiology

Is lebocin-like protein an antibacterial peptide that specifically functions in the midgut of *Spodoptera litura*?

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Lebocin is one of pro-rich antimicrobial peptides found in several Lepidoptera insects. By expressed sequence tag (EST) analysis, we found that a lebocin gene was highly expressed, particularly in the prepupal midgut of Spodoptera litura. Two full-length S. litura lebocin cDNAs (Sllebocin 1 and 2) were cloned and they are 666 and 929 bp in length, encoding 35 and 33 amino acid residues, respectively. Transcripts of these two lebocins were detected in midgut, epidermis, fat body, hemocytes and Malpighian tubules. The expression levels of the genes were much higher in the prepupal, pupal and adult stages than in the larval stage. No expression was detected in the egg. As compared to the antimicrobial peptides attacin, cecropin, defensin gallerimycin and lysozyme, Sllebocin 2 had the highest levels of expression at the pupal stage. 20-hydroxyecdysone induced the expression of these two Sllebocin transcripts. Both Gram-positive and Gram-negative bacteria induced the expression of the S. lutura lebocins in 6th instar larvae. These results suggest that in addition to antibacterial activity, the lebocins might play an un-identified role in the midgut during larval?pupal metamorphosis. This project is supported by grants from the Chinese National Natural Science Foundation (31071981) and the National High-Tech Research and Development Program (2011AA10A204).

Keywords:*Lebocin, immune respons, ecdysone, midgut, Spodoptera litura*

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S302M06

Insect Immunology, Physiology and Neurobiology S302

Drosophila immune responses against entomopathogenic nematodes

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Entomopathogenic nematodes (EPN's) of the genera Heterorhabditis are obligate and lethal insect parasites. In recent years they have been used increasingly as biological control agents. Infective juveniles occur free living in the soil and are capable of seeking out hosts and penetrate them through the cuticle or natural orifices. EPN's are symbiotically associated with bacteria of the genera Photorhabdus. The bacterial symbionts are essential to kill the host (within 24-48 hours) and digest host tissues. Drosophila larvae are more resistant to nematode infection than Galleria mellonella, but both can be used as natural infection model. The tripartite model (Drosophila, nematodes, bacteria) was recently established and used to show an immune function for transglutaminase, a conserved clotting factor. In this study we optimized the use of Drosophila as hosts and used different Drosophila mutants or RNAi lines with defects in clotting or other branches of the immune system. We demonstrated a protective function during nematode infection for known clotting substrates and for a phospholipase, while Toll an Imd immune pathways on their own are not required during nematode infection. The phenoloxidase cascade cooperates with clotting, but is not efficient again nematodes itself. In conclusion, we show that the Heterorhabditis/Photorhabdus infection model is suitable to identify novel regulators of innate immunity.

Keywords:*insect immunity, clotting, wound closure*

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Cotesia congregata bracovirus (CcBV) induces apoptosis of parasitized host plasmatocytes

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Cotesia congregata (Hymenoptera: Braconidae) develops as a gregarious endoparasitoid into larvae of the tobacco hornworm Manduca sexta (Lepidoptera: Sphingidae). The parasitoid wasp has evolved strategies of virulence using a viral obligatory symbiont, Cotesia congregata bracovirus (CcBV). CcBV particles are produced by specialized cells of the wasp ovaries and are injected along with the eggs into the host body. Previous studies showed that CcBV is essential for wasp's parasitism success and inhibits host cellular immune responses. However genes involved in this regulation, their targets and mode of action are largely unknown. We have characterized the different mechanisms involved in immune evasion of wasp eggs by cell imaging and found that the major phenomenon occurring after wasp oviposition was cell death of host plasmatocytes induced by apoptosis (as shown by detection of DNA fragmentation). The expression of some M. sexta genes specifically involved in apoptosis has been monitored in fat body cells and hemocytes: up-regulation was identified in both tissues. In addition three CcBV genes sharing sequence similarities to known virulence genes from other bracoviruses were shown to be expressed in these tissues making them good candidates for functional studies. Cell imaging approaches allows drawing a more accurate view on immune disruption during parasitism which together with high throughput expression analyses should allow an in depth understanding of the mechanisms involved.

Keywords:*polydnaviruses, apoptosis, immune disruption*

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S303TU01

Insect Immunology, Physiology and Neurobiology

Nutrition and ageing in *Drosophila melanogaster*

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Approximately 75 years ago it was reported that a moderate reduction in food intake without malnutrition extended the lifespan of rats. This intervention, known as calorie restriction or dietary restriction, has since been shown to be effective in enhancing lifespan in a range of organisms from yeast to primates and yet neither the dietary effectors nor the molecular mechanisms have been conclusively identified. We have been using Drosophila melanogaster to examine the mechanisms behind the dietary restriction effect. Our data clearly demonstrate that extremely small modifications to dietary amino acid balance can recapitulate the lifespan effects of wholesale food restriction. We have therefore been interested in further characterising these manipulations with the aim of uncovering their mechanism of action. I will present evidence for a rational approach to defining what constitutes a balanced diet for D. melanogaster and explain how it can clarify a great deal of work that has been undertaken to understand the mechanisms of dietary restriction.

Keywords:*ageing, nutrition, Drosophila melnogaster, amino acids*

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A fructose receptor functions as a nutrient sensor in the *Drosophila* brain

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Internal nutrient sensors play important roles in feeding regulation, yet virtually nothing is known about their molecular nature. Here we show by using Ca2+ imaging and behavioral experiments that the Drosophila gustatory receptor GR43a functions as a fructose taste receptor and a internal nutrient sensor. Fructose is a low abundant hemolymph sugar whose concentration increases several fold in response to nutritious carbohydrate consumption. Feeding experiments indicate that expression of GR43a in the brain is sufficient to detect nutritious carbohydrates. Moreover, the GR43a expressing brain neurons promote feeding in hungry flies and suppress feeding in satiated flies, suggesting opposite roles in the regulation of food consumption. This notion is supported in an odor-conditioning assay, which shows that artificial activation of GR43a brain neurons is perceived as pleasant in hungry, but unpleasant in satiated flies. Thus, our studies have identified a nutrient sensor in the brain that assigns valence to feeding experiences in a satiation-dependent manner.

Keywords:*nutrient sensor, Drosophila, brain, gustatory receptor 43a, feeding behavior*

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Central neurons modulating feeding motor rhythms in *Drosophila*

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Drosophila has been a powerful genetic model for studying different types of behaviors. Numerous elegant studies have combined genetic, behavioral and optical techniques to study pheromone control of mating, chemosensory signaling and olfactory learning. For feeding behavior, which has attracted much recent interest, most of the studies have been in the context of response to external olfactory/gustatory cues, or to internal hunger states. By contrast, very little is knowns on the central processes that control the motor circuits of these behaviors. We have combined electrophysiological and genetic approaches to analyze the feeding motor circuits in the Drosophila larval central nervous system. Neuronal activity of selected neurotransmitter and neuropeptide expressing cells was manipulated by temperature-inducible and optogenetic tools. We identified central serotonergic neurons that set the motor rhythm frequency of pharnygeal pumping. Axon tracing with photoactivatable GFP and live calcium imaging revealed rhythmically active glutamatergic and serotonergic neurons that send projections to the feeding apparatus and the enteric nervous system. Glutamatergic clusters comprise the feeding motor neurons and show neuritic arborizations in the subesophageal ganglion (SOG). A novel cluster of four serotonergic cells arborize in an overlapping SOG region and direct the activity of nerves that project to the alimentary tract. These results indicate that central serotonergic system coordinates multiple neural circuits underlying food intake and digestion.

Keywords:*glutamate, serotonin, feeding behavior, motor pattern, central neuronal circuits*

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S303TU04

Insect Immunology, Physiology and Neurobiology

The hungry fly larvae: strain-specific phenotypic and nutrient signaling responses to varying food

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Food nutrient content can have profound effects on organismal traits ? human obesity is perhaps the best-known example. However, genetic differences between individuals may modulate phenotypic responses, even when individuals eat nutritionally similar foods. In this study we use Drosophila melanogaster to study how variation in food protein-carbohydrate content interacts with natural genetic variation to affect developmental processes and gene expression patterns. We conducted two sets of experiments, using fly larvae. In our first experiment we reared our three fly strains on three diets that had optimal protein-carbohydrate ratios, but three different nutrient densities (ND). Performance responses (survival, mass gain, developmental time, lipid composition) were consistent across strains, and consistently improved as nutrient density increased. We observed numerous ND-by-strain interactions with respect to nutrient signaling responses, although foxo, Ilps, and InR responses showed decreased expression as nutrient densities decreased. In our second experiment our three fly strains were reared on one of four diets with different protein-carbohydrate ratios. Survival for two of our fly strains was similar across the four diets, but survival of the third strain dropped as the protein-carbohydrate ratio of the diet increased; this strain also appeared to be obesity-resistant on carbohydrate-biased treatments. Nutrient signaling responses generally showed strong diet-by-strain interactions. However, TOR signaling consistently decreased as the protein-carbohydrate ratio increased, while slif signaling was strain specific. Collectively our results provide unique insights, both at the phenotypic and gene expression level, about how food protein-carbohydrate quantity and quality affects physiological processes, including the contribution of genetic variation.

Keywords:*Drosophila, Nutrition, Protein, Carbohydrates, Genetic Variation*

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S303TU05

Insect Immunology, Physiology and Neurobiology

Evolving obesity resistance and metabolic-thrift in the genetic model organism - *Drosophila melanogaster*

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Understanding the inheritance of an obesity-prone metabolic phenotype at the molecular scale (that of genes and their regulation) could yield therapies, and inform preventative health care. Taking advantage of their short generation time, annotated genome, and the remarkable conservation of metabolic physiology among metazoans, we have used the model organism Drosophila melanogaster to begin testing how nutritional phenotypes evolve in response to changing nutritional environments. The aim has been to use laboratory selection experiments to establish the extent to which populations track shifts in their nutritional-environment, to discover the contribution of population genetic and epigenetic effects, and to use nutrigenomics to reveal molecular pathways mediating these responses.

Keywords:*transcriptome, obesity-resistance, Drosophila melanogaster, thrifty phenotype, evolution*

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S303TU06

Insect Immunology, Physiology and Neurobiology

The honeybee: A sugar junkie

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Eusocial bees are important pollinators of native and agricultural plants worldwide, but we know surprisingly little about their nutritional needs. Recent applications of the Geometric Framework to study the nutrition of adult workers have shown that adult honeybees are relatively protein intolerant: they die faster on high protein diets, and the rate at which this occurs depends on the kinds of protein they are fed. Here, I will report recent work from my lab that examines the physiological basis of protein intolerance in adult worker honeybees. We convincingly show that queenless adult workers confined to artificial liquid foods composed of sucrose and the 10 essential amino acids go through two phases of adult life: for the first week, they can tolerate a much higher amount of protein, whereas after this week, their protein tolerance sharply decreases. All adult workers we examined regulated their intake of the solutions to a carbohydrate target without regard for protein ? even if they were more likely to die from eating protein. We also show that the bee's gustatory sensitivity to solutions containing amino acids corresponds to protein tolerance during both phases. I will also compare these findings to other work from my lab investigating the nutritional requirements of another eusocial bee : the adult worker bumblebee.

Keywords:*nutrition, social insects, honeybee, amino acid*

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Nutritional limitation of food chain length

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Recent work has shown that the nutritional composition of food items can have important consequences for the growth and reproduction of predators and that some predators will tightly regulate their diet. Predator diet regulation could have important consequences for the structure and function of food webs depending upon the nutritional composition of prey at different trophic levels. We examined the consequences of predator nutrition for food chain length in terrestrial arthropod communities. We first surveyed the nutritional composition of arthropods at different trophic levels. In general, arthropods at higher trophic levels had higher concentrations of protein and lower concentrations of lipid in their bodies than arthropods at lower trophic levels. Feeding experiments with herbivorous and predatory prey indicated that these differences in whole-body nutrients reflect differences in the nutrients that predators ingest when feeding on arthropods. Finally, we examined short-term nutrient assimilation of consumers at different trophic levels and found that herbivores primarily gained lipid, primary carnivores gained both lipid and protein and secondary carnivores primarily gained protein. Our results suggest that non-protein energy becomes increasingly limited at higher trophic levels and that, as a consequence, predators may need to feed down the food chain (e.g., on herbivores) to maintain adequate intake of non-protein energy (e.g., lipid). Hence, diet regulation of carnivores may be an important factor limiting food chain length.

Keywords:*nutrition, predator, lipid, protein, food chain length*

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Understanding social behaviour through nutrition: Collective decisions in fruit flies

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In recent years, research on collective animal behaviour has revolutionized the study of social behaviour, identifying how simple interactions between individuals mediate complex group-level phenomena in a wide diversity of species. The improved understanding of the mechanisms underlying these social behaviours also has raised novel explanations for their evolution. Using the Geometric Framework of nutritional ecology, we will illustrate how nutrition regulates social interactions in the gregarious fruit fly Drosophila melanogaster. Free flying adults were observed foraging on chemically defined food sources varying in their protein to carbohydrate ratio. When given a choice between a nutritionally balanced food (optimal P:C ratio) and a nutritionally imbalanced food, grouped flies selected the optimal food source more accurately than isolated conspecifics, an emergent property of swarm intelligence in animal groups. When food sources were imbalanced but nutritionally complementary, grouped flies synchronised their spatio-temporal distribution, allowing them to balance their diet collectively by visiting foods sequentially. We developed an agent-based model to explore the collective behaviour of flies and their adaptive values in complex nutritional environments. Our findings highlight the regulatory role of nutrition in the emergence of social behaviour in 'simple' insect groups and pave the way for further examination of the impact of nutritional constraints on social evolution using novel and empirically testable predictions arising form nutritional ecology.

Keywords:*Drosophila melanogaster, Geometric Framework, collective decision, behavioural synchronisation, social evolution*

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S303TU09

Insect Immunology, Physiology and Neurobiology

Nutritional immunology: A multi-dimensional approach

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Nutrition is critical to immune defence and resistance to pathogens, with consequences that affect not only the health, welfare and reproductive success of individual organisms, but also have profound ecological and evolutionary implications. Considering the complex nature of nutritional immunology, we argue that a description of the network of interactions that define the relationships between nutrition, immune function, infection, and microbiota is essential to provide a more comprehensive and robust understanding of the key determinants of the outcome of host-pathogen interactions. Using the Geometrical Framework for nutrition, we measured these relationships using Drosophila melanogaster as a model.

Keywords:*Geometric Framework, Immunity, Microbiota, Nutrition, Pathogens*

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Variety is the spice of life: a 21st century reappraisal of dietary mixing in a generalist insect herbivore

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Generalist insect herbivores face complex challenges when feeding. Aside from encountering variation in nutrient content, both within and between plants, herbivorous insects regularly encounter nonnutritive plant compounds. Furthermore, different plants can contain different types and/or amounts of plant allelochemicals. The challenge, therefore, is to balance the need to eat to match nutritional requirements against limiting the intake of potentially harmful toxins. Work by Bernays and her colleagues in the early 90s demonstrated that generalist herbivores often benefited from eating a mixed diet, although mechanistic reasons for this enhanced growth could only be speculated. In this study, we reared early instar nymphs of the desert locust (Schistocerca gregaria) on two host plants (Schouwia purpurea (Brassicaceae, containing high levels of glucosinolates) and Hyoscyamus muticus (Solanaceae, containing high levels of tropane alkaloids)) occurring in its native range. Performance, measured in terms of survival and days to molting, was best for nymphs on the mixed plant treatment, relative to the single plant treatments. We then used a custom microarray approach to reveal novel insights into the benefit of diet mixing. The transcriptional responses of S. gregaria on the different treatments show markedly unique gene expression patterns. We highlight some of the key differences, and discuss the power of using molecular approaches to better understand the functional benefits of diet mixing in generalist insect herbivores.

Keywords:*Diet mixing, Allelochemicals*

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Polygalacturonase activity in phytophagous beetles: One way to utilize plant cell wall polysaccharides

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Primary plant cell walls are composed of approximately 90 % polysaccharides such as cellulose, hemicellulose and pectins. To utilize this massive carbon source the fibrous wall has to be deconstructed. Cellulolysis in the guts of termites is the text book example for carbohydrolytic activities in herbivorous insects. It has been shown that not only their gut microbes but termites themselves contribute to cellulose digestion by expressing cellulases. In addition, due to massive genome and transcriptome sequencing, the amount of insect putative plant cell wall degrading enzymes (PCWDEs) in general increases rapidly. Nevertheless, most characterized PCWDEs are of microbial origin and little is known about carbohydrolysis and the underlying enzymatic machinery encoded by herbivorous insect genomes. Here we report the ability of the leaf beetle Phaedon cochleariae to degrade the pectin polygalacturonan. Initial assays with their gut fluid demonstrated the presence of polygalacturonase (PG) activity. By transcriptome analysis nine putative PG encoding sequence tags could be identified. After cloning and heterologous expression followed by zymogram and agarose-diffusion assays, three of them turned out to be active PGs. Analysis of the gut fluid proteome verified the three active PGs and one inactive being present in the gut of Phaedon. To get insights in the role of PGs with respect to nutrition and herbivore host-plant interaction in general, we investigated their expression pattern and tested the direct interaction between PGs and host plant produced PG inhibitory proteins, as it is known that those act as plant defense against phytopathogenic microbes.

Keywords:*plant cell wall, polygalacturonase*

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Insect Immunology, Physiology and Neurobiology

Isothiocyanate metabolism in lepidopteran herbivores

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Isothiocyanates are the principal defensive compounds of plants in the order Brassicales, being formed from glucosinolates when plant tissue is damaged, for instance by chewing herbivores. Isothiocyanates have consistently been shown to exert negative impacts on insect herbivores in terms of growth rate, developmental time and survival. However, several lepidopterans are specialised to feed on Brassicales. The key mode of adaptation in these insects is thought to be the avoidance of isothiocyanate formation by different biochemical strategies. A number of generalist lepidopterans occasionally also feed on Brassicales, yet it is not clear how they cope with isothiocyanates. Against this background, we investigated how larvae of the generalist lepidopteran Spodoptera littoralis metabolise isothiocyanates using [14C]-labelled 4-methylsulfinylbutyl glucosinolate as a tracer. The major metabolic products of the corresponding isothiocyanate were glutathione conjugate derivatives. Thus, S. littoralis did not avoid isothiocyanate formation, but instead metabolised and excreted these compounds. Additional studies revealed that this noctuid could transform a variety of different isothiocyanates to their corresponding glutathione conjugates. We are currently investigating the role of glutathione-S-transferases in forming these metabolites. Unexpectedly, a large proportion of the labelled isothiocyanate were excreted unaltered, raising further questions regarding the mechanism of toxicity of isothiocyanates and their possible in vivo targets. We are hence exploring the biochemical basis of isothiocyanate toxicity in S. littoralis. Results from medical studies suggest that oxidative stress, mediated for example through glutathione depletion, may play a role in the observed toxicity, a possibility we are currently exploring in different generalist insect herbivores.

Keywords:*isothiocyanate metabolism, generalist lepidopterans*

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Insect Immunology, Physiology and Neurobiology

Leaf-miner insects modulate nutritional intake through an unexpected association with endosymbiotic bacteria

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Nutritional requirements of animals are multidimensional and change qualitatively and quantitatively over time. In parallel, the nutritional environment is frequently highly variable both in space and time and food sources are composed of a wide array of nutrient molecules in various ratios and concentrations combined with non-nutritive molecules. Finally, plants are known to rapidly respond to herbivore attacks by reconfiguring their metabolism in order to reduce herbivore food acquisition. As a consequence, individuals need to adopt specific pre- and post-ingestive regulatory systems to match their energetic requirements. In the context of nutritional ecology of insects, endophagous organisms are peculiar for several reasons: first, most leaf-miner and gall-inducer arthropods display high levels of fidelity to particular host-plant species. Second, their concealed habit in a confined environment prevents most endophagous larvae from switching between various plants or plant species during their development. As a consequence, while endophagous insects by their feeding habit secure their nutrition and shelter, they also have to adopt specific feeding strategies allowing them to meet their energetic requirements, to face variations in food composition and nutritional needs, and to counteract plant defensive mechanisms. Our results on the Malus domestica/Phyllonorycter blancardella plant-leaf mining system show the ability of this leaf-miner caterpillar to manipulate its host plant in order to generate a microenvironment with all the nutrient supply needed for its survival and a decrease in plant defense compounds within the mined area. Insect endosymbiotic bacteria play a primary role in this manipulation of the host plant physiology.

Keywords:*plant insect interaction, plant manipulation, endosymbiont, nutritional homeostatis*

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Putting a complete nutrient budget to the acid test - and using it

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We completed and tested a complete dynamic nutrient budget over the lifetime of an animal, the parasitic wasp Eupelmus vuilletti (Hymenoptera), in absence and in continuous presence of hosts using colorimetric essays and respirometry (CO2 & 02). This insect uses hosts, a bruchid beetle, for both oviposition and food. The nutrients are classified as proteins, carbohydrates and lipids. The model encompasses a rather large number of experimentally estimated parameters. We obtained good quantitative predictions in the absence of hosts and good qualitative predictions with continuous access to hosts. The time varying host-feeding & oviposition occurrences as well as fluctuating respiration and reserve uses all lead to highly dynamical and counterintuitive trends sustaining the animal' homeostasis. Given it good behaviour and predicting abilities over many data sets collected within a decade long period, we are now possessing a firmly based, robust model. The model was then used to explore nutrient and energy dynamics in a fluctuating environment. We varied experimentally the access to hosts for oviposition and feeding over the animal lifetime, and quantified the protein contents of females. The model was able to rank correctly the proteins content of females in different treatments of host availability. The particularly vexing problem of the overriding impact of teneral conditions as well as possible extensions of the model will be presented.

Keywords:*complete budget, respirometry*

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Plant quality is more than just nutrients: Insect herbivores can choose microclimates to achieve nutritional homeostasis

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Temperature and nutrition interact to affect the life histories of ectotherms. These interactions are generally believed to be mediated via metabolic rate, rather than by changes in the rates and ratios at which particular nutrients are digested, absorbed and utilized. We show that the rates and balance of protein and carbohydrate absorbed from particular host plants by a model herbivore, the locust, is temperature dependent. Furthermore, locusts were able to choose body temperatures so that protein and carbohydrate were differentially absorbed to redress experimentally imposed nutrient imbalances. Hence, 'diet quality' is temperature sensitive and insect herbivores can manipulate this relationship to their own ends. These findings suggest micro-climatic effects may be of more importance than previously appreciated for understanding the consequences changed environmental temperatures and microclimate on animal-plant interactions.

Keywords:*nutrient balance, dynamic thermoregulatory behaviour, behavioural plasticity, learning*

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Insect Immunology, Physiology and Neurobiology

Interplay of nutrition and temperature affecting food intake and performance in insects: A case study from caterpillars

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Temperature and nutrition are the two most prominent environmental determinants of fitness in insects. However, it remains largely unexplored how variations in food nutrient quality mediate the relationships between ambient temperature and various aspects of insect performance with respect to behavior, physiology and life-history. The present study investigates the separate and combined effects of ambient temperature and nutrient imbalance on key components of fitness, food intake and selection, and post-ingestive nutritional physiology in two species of caterpillars, Spodoptera exigua and S. litura (Lepidoptera: Noctuidae). In the no-choice test, caterpillars were confined to one of six nutritionally imbalanced diets that varied in protein and carbohydrate content (P:C = 42:0, 35:7, 28:14, 21:21, 14:28, 7:35) under five different temperatures ranging from 12 to 42 °C. Caterpillars grew and ate faster when temperature rose gradually up to 34°C but suffered high mortality and reduced efficiencies of nutrient utilization. Response surfaces that relate temperature, nutrition, and larval performance showed that optimal temperature for growth and survival differed considerably and that the thermal sensitivities of key life-history traits were constrained by dietary imbalance, suggesting significant temperature-by-nutrient interactions. When allowed to select their preferred diet, caterpillars in the warmer thermal regime (above 30°C) preferentially selected carbohydrate-rich diet whereas those in the cooler (below 30°C) consumed the equal ratio of protein to carbohydrate. Such temperature-induced shift in diet preferences allows caterpillars to supplement extra energy that is required for fueling elevated metabolic rates when living and growing in the warmer thermal environment.

Keywords:*caterpillar, diet selection, nutrient regulation, protein:carbohydrate balance, temperature*

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Insect Immunology, Physiology and Neurobiology

Low plant nitrogen content and high population density enhance migratory characters in a polyphenic locust

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Locusts present an impressive and well-studied example of phenotypic plasticity in which population density regulates a variety of behavioral, morphological, and physiological traits that often culminate in migratory swarms. Oedaleus asiaticus, a north Asian locust, has outbreaks and migratory swarms comprised predominantly of brown morphs. Heavy livestock grazing promotes outbreaks by lowering plant nitrogen content and likely lowering the protein:carbohydrate ratio to the optimal range for this locust. However, it is unclear if a change in plant quality can modulate the effect of density on phase characteristics. We found that locusts reared at high population density and fed low-N plants (i.e. plants that are high quality for O. asiaticus) had the most enhanced migratory characteristics while locusts fed high-N plants (i.e. deleterious for this locust) consistently had decreased expression of migratory characters. These results do not support existing paradigms that poor-quality resources increase the expression of migratory phenotypes. We then compared feeding habits of the brown (outbreak) and green (non-outbreak) morphs. There was no difference in plant preference nor protein:carbohydrate intake target ratio; however, when confined to diets extreme in their protein:carbohydrate ratios, green locusts decreased consumption and brown locusts maintained a similar consumption rate on all diets. From these results we infer that the green morph strategy may be most beneficial for sedentary insects confined to limited food choices to avoid long-term deleterious effects of consuming an unbalanced diet, while the brown morph strategy of being more willing to consume suboptimal foods may be most beneficial for roaming insects.

Keywords:*phenotypic plasticity, locust*

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Insect Immunology, Physiology and Neurobiology

Nutrient regulation strategies in wing polymorphic crickets reflect life history allocation trade-offs

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An organism's life history and phenotype should be intimately linked to optimal nutrient regulation strategies, yet actual demonstrations of such connections are rare. Here, we show how a well-studied trade-off between dispersal and reproduction in the wing-polymorphic cricket Gryllus firmus is tied to differences in feeding behavior. In a "choice" experiment, we offered female crickets selected either for dispersal (long-winged individuals, LW) or reproduction (short-winged individuals, SW) access to two imbalanced, but complementary foods that varied in their protein and digestible carbohydrate content. In a second, "no-choice" experiment, we confined crickets to one of five imbalanced foods that were either protein- or carbohydrate-biased. In both cases, we measured crickets' food intake, mass gains, and lipid profiles. LW crickets allowed to self-select between foods chose a diet that was more carbohydrate-biased than that selected by SW crickets, resulting in higher lipid gains but equal mass gains. When confined to single foods, with a range of protein-carbohydrate ratios, SW crickets over-ate whichever nutrient was available in excess; these crickets gained more mass, yet had similar lipid profiles compared to the LW crickets. These patterns show that in this species feeding behavior directly influences the dispersal/reproduction trade-off, elucidating how behavioral and physiological processes drive the expression of this phenotype.

Keywords:*nutrition, carbohydrate, protein, cricket, life-history*

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Introduction

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Insect saliva helps the feeding and digestion of food in various ways. Physically, saliva helps to dissolve solid food, as in the house fly, or to seal the mouthparts of feeding aphids when tapping their plant hosts vessels. Biochemically, saliva has enzymes that assist the digestion of the ingested food, or the prey itself in the case of saliva injected by predators. Pharmacologically, the saliva of insects feeding on living organisms can modify their physiology to increase the success of the meal, by injection of paralytic substances by predators or by injection of anti-clotting agents by blood feeding arthropods. Saliva of vector arthropods is also the vehicle for pathogen delivery and/or modifies the site of pathogen injection and accordingly becomes a target for vaccine interventions. Saliva of blood sucking insects consistently have anti-clotting, anti-platelet, vasodilatory and immunomodullatory components. Recent transcriptome analysis indicate that the salivary potion of these organisms contain 50-125 polypeptides in insects and several hundreds in ticks. Possibly due to the host immunological response, these proteins are at a very fast pace of evolution and many salivary proteins have new folds and unknown function. Recent advances in our laboratory related to the discovery of the function of these novel proteins will be presented.

Keywords:*salivary vaccine plant-insect*

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Vector saliva as a vaccine target to prevent infectious diseases

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In vector-borne diseases the contribution of vector saliva in parasite establishment in mammalian hosts is largely ignored. We tested the hypothesis that vector salivary proteins can modulate the host immune response to the parasite and protect the host against infection. Injection of a recombinant sand fly salivary protein without adjuvant induced a long-lasting cellular immunity in mice and conferred powerful protection against the Leishmania parasite delivered by sand fly bites. This protection is antibody independent and is abrogated by depletion of CD4+ T cells. This is the first evidence that a vector protein can confer protection against sand fly transmitted parasites. We propose that this salivary protein is an immunomodulator that can drive an early and controlled response to a sand fly-transmitted virulent Leishmania somewhat mimicking "leishmanization"- induced protection but without its associated pathology.

Keywords:*sand fly saliva, leishmaniasis, protective immunity, cellular immune response*

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Mosquito salivary glands as vaccine delivery targets

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We have established a robust salivary gland-specific transgene expression in transgenic (TG) Anopheles stephensi mosquitoes. Under control of a newly isolated salivary gland-specific promoter, robust expression of DsRed tetramer (tDsRed) protein was achieved in the cytoplasm (cytoplasmic form) of the salivary gland cells of distal-lateral lobes, where the sporozoites invade preferentially. This salivary gland-specific promoter drives the expression of Anopheline Anti-Platelet Protein (AAPP), which exhibits a strong and specific inhibitory activity toward collagen-induced platelet aggregation. Using the AAPP promoter, we have recently generated a TG mosquito which secrets a foreign protein as a component of saliva during blood feeding. The foreign protein is SP15 fused to DsRed monomer protein (mDsRed-SP15). The SP15 antigen has originally been identified from sand fly saliva as a leishmania vaccine candidate antigen. We observed that the mDsRed-SP15 protein was accumulated in the cavity and duct of the salivary gland. Interestingly, mice repeatedly bitten by the TG mosquitoes had anti-SP15 antibodies, indicating injection of the mDsRed-SP15 protein during blood feeding. These results may suggest a possibility that a TG mosquito expressing a vaccine antigen in the saliva can delivery its vaccine to the hosts (known as "Flying vaccinator"). However, we are aware that the concept of the flying vaccinator is not in itself going to be a practicable method of disease control. So, the present study is a model case and we intend to apply this system for elucidation of saliva-malaria sporozoite interaction.

Keywords:*anopheline mosquito, saliva, vaccine, leishmania*

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Vector salivary antigens as epidemiological markers

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The study of human-vector immune relationships could allow several applications for the control of vector-borne diseases. Indeed, some salivary proteins from blood-feeding arthropods could induced a specific immune responses in human populations exposed to arthropod vectors bites. So, why do not use the immunogenic properties to develop of biomarker of human bites exposure? The case of Anopheles-Plasmodium complex and similar approach for Aedes-borne diseases (arbovirus) will be focused here. One hypothesis is that human immune response and especially antibody (Ab) response to whole saliva of mosquito could be an epidemiological biomarker of human exposure to vector bites. In the objective to increase the specificity to vector exposure, the second step was to identify salivary proteins i) specific to Anopheles/Aedes genus and ii) antigenic in individuals exposed to malaria or arbovirus diseases. First, the identification of antigenic salivary proteins of mosquito by an immuno-proteomic approach was assessed. The second step was to design peptide sequences, from one selected mosquito salivary protein using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with other arthropods/organisms. For malaria, the specific IgG Ab levels were then evaluated in African children in different context of malaria. From five peptides, only one peptide (gSG6-P1) presented all criteria to be an optimal candidate biomarker for evaluating human exposure to An. gambiae and An. funestus bites and interestingly for evaluating the efficacy of vector control. The same strategies developed for measuring human exposure to Aedes will be also presented.

Keywords:*biomarker - antibody - salivary proteins - vector-borne diseases*

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S304TU05

Insect Immunology, Physiology and Neurobiology

The role of salivary anti proteases in tick feeding and host manipulation

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We demonstrated that tick cysteine protease inhibitors (cystatins) are essential for feeding success of Ixodes scapularis, the vector of Lyme disease agents in the eastern and central USA. One of these inhibitors, named sialostatin L2, facilitates both Borrelia burgdorferi and Anaplasma phagocytophilum establishment in the vertebrate host, uncovering that upon host colonization two different tick-borne pathogens exploit an essential protein for tick feeding success. The pharmacological action of an I. scapularis cysteine protease inhibitor, named sialostatin L, has the potential to alleviate the symptoms of autoimmune encephalitis and allergic asthma in mice. Tick saliva also contains serine protease inhibitors which target various vertebrate host proteases; we already described the structural basis for the unique target specificity and pharmacological properties of a serine protease inhibitor of the serpin superfamily, named IRS-2. We propose that the role of proteolysis regulation by tick salivary secretion is prominent in the tripartite interaction between ticks, the vertebrate hosts and tick borne pathogens. Hematophagy has resulted in the adaptation of the ticks to the vertebrate hosts through the inclusion in their salivary secretion of substances with potent pharmacological action. We currently test whether ticks can serve as model organisms for the discovery in their salivary glands of bioactive molecules with unique molecular targets and mechanism of action in the host. Beyond investigating the structural basis for their action we also test the potential of these molecules for future drug development in animal models of various diseases.

Keywords:*protease inhibitors, cystatin, serpin, tick saliva, arthropod vector-vertebrate host-pathogen interaction, tick-borne pathogens, pharmacological action, experimental asthma*

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Insect saliva in plant insect interactions

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Piercing-sucking hemipteran insects, such as aphids, display typical feeding behaviours suggesting active suppression of plant defence responses (1). Functional genomics of the green peach aphid Myzus persicae resulted in the identification of effectors that are present in the aphid saliva and modulate plant processes (2). Silencing of these effector genes by plant-mediated RNA interference (RNAi) in M. persicae (3) reduces this aphid's fertility on plants, while M. persicae has enhanced fertility on transgenic Arabidopsis plants that express the M. persicae effector genes. Interestingly, M. persicae fertility did not increase on plants that express effectors of a closely related aphid species, the pea aphid Acyrthosiphon pisum, that are highly similar in amino acid sequence to the M. persicae effectors. A. pisum is a legume specialist, and does not use Arabidopsis as a host. Thus, aphid effectors have adapted to modulate plant processes to the benefit of aphids in specific plant hosts. We also have evidence that M. persicae suppresses the plant basal immunity, i.e. Pathogen-Associated Molecular Pattern (PAMP) Triggered Immunity (PTI). We have identified plant receptors involved in the induction of PTI to aphids and characterized an aphid effector that suppresses PTI. This is the first evidence that an immune receptor is involved in host perception of an insect pest. Altogether, our work provides new avenues for research to improve insect pest control. (1) Bos and Hogenhout (2011). Curr. Opin. Plant Biol. 14: 1-7. (2) Bos et al. (2010). PLoS Genetics 6: e1001216. (3) Pitino et al. (2011). PLoS One: e25709.

Keywords:*PAMP-triggered immunity (PTI), Virulence proteins, Effectors, Aphid saliva, Immune receptors*

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Insect Immunology, Physiology and Neurobiology

Proteomics of aphid salivary proteins

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Relations between insects and plants are complex. By both direct and indirect mechanisms, locally or systemically, plants defend themselves toward a diversity of insect pests. To induce these defense pathways, elicitation signals have to be perceived by plants, including both mechanical and biochemical factors according to the pest feeding behaviour. Until now, chewing feeders inducing large plant damages were the most studied models for plant defense elicitation phenomena. Several elicitors were already identified : the volicitin in Spodoptera exigua, a glucose oxidase in Helicoverpa zea and a β-glucosidase in Pieris brassicae. A specific signature hypothesis was oftenly cited to explain the diversity of elicitation compounds in this range of insect saliva. Other herbivores, such as aphids are also largely distributed on a diversity of host plant with significant specificities. We performed proteomic tasks to characterize potential elictors in several aphid species but also in different aphid clones from the same species according to different pattern of endosymbionts. Complementary approaches were performed to identify and characterize aphid salivary proteins : separation by one and two dimension electrophoresis coupled with Maldi-Tof identification, use of nano-liquid chromatography coupled with mass spectrometry (ESI MS-MS), functional tests on whole plants or cell culture using aphid saliva (assessing the oxydative burst, pH changes, plant volatile patterns and proteomic changes in plant materials). One of the interesting protein found in aphid saliva was a glucose oxydase. This finding was discussed in relation to the specific signature hypothesis in plant-insect relations and potential perspective in aphid pest control.

Keywords:*aphid, elicitor, plant defense*

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Function and antigenicity of mosquito salivary proteins

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Substantially improved knowledge of mosquito salivary repertoires has been achieved in the last decade through transcriptome and expression/functional studies. Nevertheless, a large number of salivary proteins are so far without a function and our understanding of the effects of mosquito saliva on host immune response and pathogen transmission/infection is still rudimentary. We recently characterized two Anopheles gambiae salivary proteins restricted to anopheline mosquitoes and involved in hematophagy: gSG6 and cE5. The first helps mosquito blood feeding by a still unknown mechanism, the second is a highly specific fast- and tight-binding thrombin inhibitor. Both proteins are immunogenic to humans and evoke different humoral responses in exposed individuals from a malaria hyperendemic area. The IgG response to gSG6 decreased with age whereas anti-cE5 IgG levels showed an opposite trend. IgG4 was the dominant anti-gSG6 IgG subclass (IgG4>IgG1), instead IgG1 was the main anti-cE5 antibody (IgG1>>IgG4). Since IgG1 and IgG4 are often considered as markers of Th1- and Th2-type responses we believe we have identified two salivary antigens with strikingly different antigenic properties: (i) gSG6, triggering a Th2-type response, with high IgG4 levels and induction of tolerance; (ii) cE5, eliciting a Th1-type response, with high IgG1 levels and no development of tolerance. The availability of salivary components triggering differential host responses is expected to help dissecting the complexity of the human immune response to mosquito saliva and to provide a deeper understanding of the potential for mosquito salivary proteins to be exploited in the fight against vector-borne diseases.

Keywords:*Anopheles gambiae, salivary proteins, humoral response, anti-thrombin*

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Role of saliva in vectors of dengue virus transmission

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Dengue virus (DENV) infection is the most prevalent mosquito-borne viral diseases in the world. Vector-mediated transmission of DENV is initiated when a blood-feeding female Aedes mosquito injects saliva, together with the virus, into the skin of its mammalian host. Understanding the role of skin immune cells in the activation of innate immunity to DENV at the early times of infection is a critical issue that remains to be investigated. The purpose of our study was to assess the contribution of human keratinocytes as potential host cells to DENV. We show that primary human keratinocytes support DENV replication with the production of negative-stranded viral RNAs inside the infected cells. In the course of DENV life cycle, we observed the activation of host genes involved in the antiviral immune responses such as intracellular RNA virus sensors. DENV infection of primary keratinocytes also resulted in up-regulation of the expression of the antiviral Ribonuclease L gene, which subsequently led to enhanced production of IFNs. We have also investigated the effect of Aedes salivary gland extract (SGE) on DENV infection in human keratinocytes. We show that Aedes SGE enhances DENV infection of human keratinocytes via the inhibition of the secretion of antimicrobial peptides and interferons by the infected cells. The results underscore the importance of Aedes saliva in the earliest events of DENV transmission.

Keywords:*Saliva, Dengue, Keratinocytes, Aedes, Arbovirus*

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S305W01

Insect Immunology, Physiology and Neurobiology

PTTH coordinates physiological and behavioral transitions during *Drosophila* development

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Prothoracicotropic hormone (PTTH) is a brain neuropeptide that regulates the timing of molting and metamorphosis in insects. PTTH activates its receptor Torso expressed in the prothoracic gland (PG), which transduces a downstream signal through the MAPK pathway to stimulate the production of the steroid hormone ecdysone. Ecdysone in turn regulates the expression of multiple genes in most larval tissues to coordinate all the physiological and morphological changes that accompany molting and metamorphosis. To date, all known functions of PTTH during insect development appear to be mediated indirectly through control of ecdysone production and release. By using Drosophila genetic tools to manipulate the function of Torso in different cell types, we have recently uncovered a novel function of PTTH that is independent of its prothoracicotropic effect on the PG. We find that PTTH acts directly on a subset of neuronal cells to regulate certain behavioral transitions that Drosophila larvae undergo prior to metamorphosis. Therefore, PTTH appears to be a master regulator of insect development via at least two parallel pathways that coordinates not only physiological and morphological changes during metamorphosis, but also at least one behavioral transition that presumably contributes to the success of this process. The significance of these independent functions of PTTH will be discussed.

Keywords:*PTTH, Torso, ecdysone, behavior*

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Expression and function of neuropeptides and their receptors in *Bombyx mori*

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The silkmoth Bombyx mori is a suitablemodel species for analysis of complex physiological and biochemical processescontrolled by neuropeptides and their receptors. To understand neuroendocrine interactions between these important regulatory molecules we determined the temporal and spatial expression patterns of all known neuropeptides and selected receptors in the CNS and peripheral endocrine organs.In situ hybridization with DNA probes combined with double/triple immunohistochemical staining revealed the presence of multiple receptors in peptidergic and aminergic central neurons as well as endocrine cells. To further examine the role of these neurons and endocrine cells, we used the baculovirus expression system for identification of neuropeptide promoters suitable for targetedexpression of selected receptors in the CNS and endocrine organs. Characterized promoters with receptors or molecular markershave been subcloned into piggyBac vectors and used for production of transgenic animals. Targeted expression of various receptors and markers revealed specific functions and unexpected neuroendocrine pathways between specific central neurons and endocrine organs. Some specific pathwaysand receptor functions will be discussed in more detail.

Keywords:*Bombyx mori*

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Drosophila short neuropeptide F (sNPF) signaling regulates lifespan, body growth, and carbohydrate metabolism through the insulin signaling

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Drosophilashort neuropeptide F (sNPF) is processed and expressed in the nervous systems of various developmental stages. Inhibition of sNPF in the nervous system shows increased lifespan, small body size, and increased blood carbohydrate level compared to the Wild-type control flies. These are typical phenotypes of mutants in the insulin signaling, which suggest that sNPF may regulate insulin signaling. Therefore, we investigated whether sNPF regulates insulin signaling in the insulin producing cells (IPCs) and insulin target tissues. After immunostaining with the anti-serum against sNPF receptor (sNPFR1) in the fly brain, we found that sNPFR1 is localized in the plasma membrane of IPCs. When sNPF was inhibited in the neurons, the expression level of the Drosophila insulin-like peptides (Dilps) gene was decreased compared to that of the Wild-type control. Likewise, sNPFR1 suppression in IPCs reduced Dilps expression. These regulations of Dilp expression by sNPF and sNPFR1 in IPCs are mediated by ERK. In the fat body, which is the homologous tissue of mammalian liver and adipocytes, sNPF mutants inhibited the activation of Akt, induced the nuclear localization of FOXO, and increased the expression of the translational inhibitor 4E-BP. It indicates that sNPF mutants inhibited the insulin receptor signaling in the fat body. These IPCs and fat body data demonstrate that sNPF and sNPFR1 regulate lifespan, body size, and glucose metabolism through the insulin signaling.

Keywords:*carbohydrate metabolism*

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Neuropeptides in the midgut

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Endocine cells in the midgut of both larval and adults of Drosophila melanogaster were identified with antisera to various neuropeptides. Allatostatins A, B and C, neuropeptide F, diuretic hormone 31, tachykinins and short neuropeptide-F (sNPF) producing endocrine cells were found. Axons immunoreactive toward antisera to short neuropeptide F and pigment-dispersing factor are also present in the midgut. The Drosophila insulin 3 gene is also expressed in the midgut, but dilp3-gal4 flies suggest that it is produced muscles, not endocrine cells.

Keywords:*midgut, NPF, sNPF, allatostatin, DH31, PDF, tachykinin*

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Updates of neuropeptide mediated regulation of ecdysone biosynthesis in the prothoracic glands of *Bombyx mori*

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The insect steroid hormone, ecdysone is important for the regulation of molting and metamorphosis. The ecdysone is predominantly synthesized in the prothoracic glands (PGs) from dietary cholesterol or phytosterols. The ecdysone biosynthesis is intricately regulated by neuropeptides. To date, four prothoracicotropic peptides (PTTH, FXPRL-amides, insulin and orcokinins) and three prothoracicostatic peptides (PTSP, Bommo-myosuppressin and Bommo-FMRFamides) have been identified in Bombyx mori. However, the whole regulatory mechanisms of ecdysone biosynthesis are not fully understood, and involvement of more neuropeptides is expected. To elucidate the entire pathway of ecdysone biosynthesis including its regulatory mechanisms, we performed a transcriptome analysis of the PGs in B. mori using next-generation sequencing, and found a G-protein coupled receptor (GPCR) mediated novel regulatory mechanism in the ecdysone biosynthesis. Quantitative analyses of the sterols in the cultured medium and PGs by liquid chromatography-tandem mass spectrometry revealed that a ligand of the GPCR markedly induced the ecdysone biosynthesis, and reduction of 7-dehydrocholesterol content in the PGs was observed. On the other hand, the ligand was not affected for the cholesterol content. In addition, we discuss the similarity and difference between this novel regulatory mechanism and PTTH mediated regulatory mechanism in the ecdysone biosynthesis. [This study was supported by the grant from Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.]

Keywords:*ecdysone biosynthesis, neuropeptide, GPCR*

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Multiple transmitter systems regulate Adipokinetic Hormone Signaling in *Drosophila*

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Adipokinetic Hormone (AKH) is the insect equivalent of mammalian glucagon as it is the primary hormone responsible for the release of stored energy. We recently identified that the AMP-activated kinase (AMPK) as a critical factor that regulates AKH secretion via modulation of AKH cell excitability. Notably, the loss of AMPK function in AKH cells leads to a partial phenocopy of AKH cell ablations, suggesting additional regulators of AKH signaling. To identify these, we performed a genome-wide screen of peptide and amine GPCRs that potentially regulate AKH signaling. Specifically, we employed an RNAi strategy to specifically target the entire complement of peptide and amine GPCRs in Drosophila. Our prediction was that the loss of receptors that function to facilitate the release of AKH would lead to behavioral phenotypes consistent with the loss or reduced AKH function (e.g., starvation resistant, no hyperactivity). In contrast, we reasoned that the loss of receptors which normally inhibit AKH release would yield opposite phenotypes (starvation sensitivity, greater amounts of hyperactivity, etc). We identified that animals expressing RNAi targeting the receptor for the Allatostatin C (AstC-R1) show increased starvation resistance. We confirmed that AKH cells express this receptor using RNAseq and single-cell RT-PCR. Furthermore, using GCaMP as a reporter for AKH cell activation, we observe that application of AstC increases AKH secretion in an energy independent fashion. We also note an epistatic interaction of AstC and AMPK, suggesting that AstC acts to modulate AMPK activity. In addition to AstC, we will present findings on other transmitter systems.

Keywords:*Adipokinetic Hormone*

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G-protein coupled receptors for regulating ecdysteroid biosynthesis in the fruit fly *Drosophila melanogaster*

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In this presentation we report that multiple G-protein coupled receptors (GPCRs) are involved in biosynthesis of ecdysteroids, the principal steroid hormones guiding insect molting and metamorphosis. During insect development, ecdysteroids are synthesized from dietary cholesterol in a special endocrine organ called the prothoracic gland (PG). Ecdysteroid biosynthesis in the PG is controlled in response to several external conditions, such as nutrition, temperature and photoperiod. This adaptive change of ecdysteroid biosynthesis results in flexible alterations of developmental timing and growth, which enable insects to survive in various harsh environments. However, it remains unclear how external information is transmitted to the PG to adaptively control the catalytic activities of the ecdysteroidogenic enzymes. To uncover essential genes involved in the precise regulation of ecdysteroid biosynthesis in the PG, we conducted a transgenic RNAi screen using the PG-specific GAL4 drivers and UAS-RNAi strains in the fruit fly Drosophila melanogaster. We found that developmental arrests were caused by PG-specific RNAi for genes encoding several GPCRs including neurotransmitter receptors. These phenotypes were rescued when the RNAi animals were fed 20-hydroxyecdysone-supplemented food. Moreover, the 20E titer and the expression levels of ecdysteroid biosynthetic enzyme genes significantly decreased in the RNAi animals. Interestingly, a subset of neurotransmitter-producing neurons directly innervated to the PG. Considering that neurotransmitters modulate target activities in response to external stimuli, we currently hypothesize that the neurotransmitters and their GPCRs play important roles in reflecting environmental conditions on ecdysteroid biosynthesis in the PG.

Keywords:*ecdysteroid biosynthesis, GPCR, Drosophila melanogaster*

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Identification of the molecular domain that confer ligand selectivity of the sex peptide receptor

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The sex peptide receptor (SPR) mediates actions of the sex peptide (SP), a male seminal fluid protein that triggers the post-mating response in females. SPR is found in almost all sequenced insect genomes and Aplysia, whereas its ligand SP is found only in handful numbers of Drosophila species, inferring its recent evolutional origin. Previously we identified myoinhibitory peptide (MIPs) as an ancestral family of SPR ligands that is highly conserved across insect species and has the same phylogenetic distribution as SPR. MIPs are potent agonists for Drosophila, Aedes, Anopheles, Bombyx and Aplysia SPRs in vitro. In contrast to MIPs, SP is potent only on Drosophila SPR (dSPR), but not on other non-Drosophilidae SPRs. To understand how dSPR evolves its dual-ligand selectivity, we setout to identify the molecular domain of dSPR responsible for SP-sensitivity, and ask it is also required for MIP-sensitivity. We divide SPR into 8 domains, and perform a systematic domain swapping between SP-sensitive dSPR and SP-insensitive Aedes SPR (aSPR). By replacing domains of dSPR with corresponding aSPR domain systematically, we identify domain number 5 (D5) of dSPR, necessary for the SP-sensitivity. In parallel we swap aSPR domains with dSPR ones and find D5 domain also confers SP-sensitivity on aSPR. Together both analyses equivocally show D5 domain is necessary and sufficient for the SP-sensitivity. Currently D5 domain is being mapped with the fine scale domain swapping. Results from this study will allow us to formulate a testable model that explains molecular mechanisms underlying dual-ligand selectivity of SPR.

Keywords:*Peptide, GPCR, Drosophila, sex peptide receptor, Reproductive behaviors*

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Neuropeptide G protein coupled receptors in *Glossina morsitans*

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The tsetse fly, Glossina morsitans, serves as a vector for protozoa of the species Trypanosoma brucei that cause African trypanosomiasis also called sleeping sickness, a parasitic disease of people and animals. The disease is endemic in some regions of sub-Saharan Africa, covering about 37 countries and 60 million people and leads to the death of ten thousands of people each year. The insects are less becoming susceptible to currently used insecticides due to increasing resistance. G protein coupled receptors (GPCRs) provide a potentially interesting target for the development of novel insecticides. We are annotating GPCRs and neuropeptides in the genome of Glossina and we are deorphanising the GPCRs for several neuropeptides, including adipokinetic hormone (AKH), allatostatins, pyrokinins, short and long neuropeptide F. Realtime PCR is used for spatial and developmental expression analysis of these GPCRs. Future experiments include reverse genetics in order to determine the physiological role of the GPCRs in Glossina.

Keywords:*Glossina morsitans, neuropeptde, G protein coupled receptor*

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S306TH01

Insect Immunology, Physiology and Neurobiology

Evolutionary plasticity of insect immunity

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The rapidly increasing number of insect species which have been subjected to genome sequencing or to transcriptomic analysis of their innate immune responses invite comparative studies. From the molecular perspective, emergence of novel proteins or protein domains is foremost associated with adaptation to abiotic and biotic environmental stressors. The tremendous evolutionary plasticity of insect immunity is highlighted focusing on effector molecules such as antimicrobial peptides (AMPs) or proteinase inhibitors which display a higher evolutionary dynamic than, for example, immunity-related signaling molecules. Increasing evidence support the hypothesis that the functions of insect AMPs can be expanded beyond their role in defense against pathogens to include the management of beneficial endosymbionts. The total number of AMPs varies considerably in insects and provides intriguing examples for immune gene expansion and loss. This plasticity is discussed in context with recent developments in evolutionary ecology. Maintenance and deployment of immune responses allocates resources from other fitness-related traits and results in trade-offs. Based on our recent studies using both model and-non-model insects I postulate that loss of immune genes can occur in insects which evolved alternative and less fitness cost demanding defense strategies such as the so-called social immunity in bees, or the chemical sanitation of the microenvironment by some beetles. On the other hand, recent studies provide evidence for diversification and functional shifts of AMPs or defense-related proteinase inhibitors in insects which solely reflect coevolution with a changing pathosphere and/or adaptations to habitats or food associated with microbial contamination.

Keywords:*Evolutionary ecology, antimicrobial peptides, immune gene expansion*

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Virulence factors produced by pathogens provide important insights into the function of the insect immune system

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Insects rely upon a well-coordinated innate immune system for protection against foreign invaders. Among the most important natural enemies of insects are parasitoid wasps that carry polydnaviruses (PDVs). PDVs do not replicate in the wasp's host but expression of PDV-encoded genes cause physiological alterations that are essential for survival of the wasp's progeny. The most important of these alterations is immunosuppression. Genome analysis indicates that PDVs encode several genes of potential importance in immunosuppression while physiological studies identify several PDV-induced alterations in host immune defenses. Collectively, these data indicate the majority of the virulence factors encoded by PDVs target the signaling pathways that regulate host immune effector responses rather than effector molecules themselves.

Keywords:*hemocyte, humoral, parasite, virus*

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S306TH03

Insect Immunology, Physiology and Neurobiology

Infection-induced physiological interaction between the mosquito circulatory and immune systems

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Mosquitoes respond to infection by mounting cellular and humoral immune responses. In the hemocoel, these responses are mediated primarily by hemocytes, which sequester and kill pathogens via phagocytic, lytic and melanization pathways. While much work has focused on the cellular biology of mosquito hemocytes, little is known about the in vivo dynamics of hemocyte-pathogen interactions in the insect hemocoel. Hemolymph currents disseminate invading pathogens to all regions of the hemocoel, and likewise, hemolymph currents propel hemocytes as they search for pathogens. This presentation will describe the temporal and spatial dynamics of hemocyte-pathogen interactions in the mosquito hemocoel, and will detail how hemocytes aggregate on the surface of the heart in response to infection. Using a novel method that stains Anopheles gambiae hemocytes in vivo, we found that mosquito hemocytes occur both in circulation and attached to tissues. Intravital video imaging then showed that infection with bacteria or malaria parasites induces the migration of circulating hemocytes to the periostial regions of the heart (regions adjacent to the heart valves), where they bind heart and alary muscles and engage in the phagocytosis of pathogens. Directed hemocyte migration to the periostial regions occurs in a time- and dose-dependent manner, with higher infection intensities inducing the aggregation of higher numbers of hemocytes. Together, these data describe the physiological interaction between the mosquito circulatory and immune systems, where hemocytes aggregate and phagocytose pathogens in the areas of highest hemolymph flow.

Keywords:*Mosquito, Anopheles gambiae, hemocyte, haemocyte, hemolymph, haemolymph, heart, bacteria, malaria, Plasmodium*

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The impact of isoprenoid precursors released by parasites and pathogens on the immune response in *Drosophila* and *Anopheles*

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Isoprenoid precursors are small, non-peptidic compounds known to induce activation and massive proliferation of human Vγ2Vδ2 T-cells, which are important in killing malaria parasites.Little is known however about their effects in invertebrates. We have investigated the stimulating properties of isopentenyl pyrophosphate (IPP) and (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) in malaria mosquito Anopheles gambiae. IPP is a universal isoprenoid precursor found in all living organisms. HMBPP is present in most eubacteria and in apicomplexan protozoa.It is the most potent Vγ2Vδ2 T-cells activator known with a bioactivity at picomolar concentrations. When HMBPP was provided in the blood meal to Anopheles gambiae,it induced the expression of immune and redox regulating genes in female mosquitoes. Also, HMBPP stimulation of A. gambiae4a3B cells, lead to activation of several signal transduction pathways that have earlier been implicated in human γδ T cell responses. HMBPP and IPP also increased 4a3Bcell tolerance to H2O2 induced oxidative stress.

Keywords:*isoprenoid precursors, gdT-cells, Anopheles gambiae,immune response, red-ox, regulation, Plasmodium falciparum*

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The endosymbiont Wolbachia as an extension of the host antiviral immune system

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Although single host-virus interactions are often studied in isolation, host organisms are typically infected by many microbe populations. Tripartite interactions between two microbes and the host can alter pathogenesis and disease outcome. In insects, the bacterium Wolbachia can protect the host against viral infection and viral-induced mortality. Drosophila is an important model system for understanding interactions between invertebrates and viruses. Remarkably, the extent of Wolbachia mediated antiviral protection in Drosophila is similar to the protection afforded by the host antiviral responses, indicating that the interaction between Wolbachia and virus has important biological implications. To explore the mechanism of antiviral protection, we investigated the importance of Wolbachia density within the insect host on antiviral protection. To do this, we used low doses of antibiotic to decrease the density of a protective Wolbachia strain in its natural host, to levels that were similar to those observed in two non-protective Wolbachia strains. Flies were then challenged with the pathogenic Drosophila C virus. It was found that when the density of the previously protective Wolbachia strain wAu was decreased to levels similar to the non-protective strains, Wolbachia-mediated antiviral protection was substantially decreased or lost completely. This data indicates that the density of Wolbachia in the insect host is important for the mechanism of antiviral protection and suggests that insects carrying a low load of Wolbachia will likely not be protected. These findings may facilitate prediction of Wolbachia-mediated protection in host-Wolbachia associations relevant for the biocontrol of insect-borne vir

Keywords:*Drosophila, virus, wolbachia, anti-viral*

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Mosquito immunity to human pathogens

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Lacking the unlimited repertoire of antibodies provided by adaptive immunity, insects largely rely on their innate immune systems, involving a finite number (~400) of germ line-encoded immune genes, for their anti-pathogen defenses. Despite this limitation, insects can control infection by a great variety of parasites, microbes and viruses through immune deployment which largely is mediated by immune signaling pathways that control expression of anti-pathogen effector molecules. We have shown that the Aedes aegypti uses its Toll and JAK-STAT immune signaling pathways to combat dengue virus infection, while Anopheles mosquitoes use their IMD pathway to control infection with the human malaria parasite Plasmodium falciparum. We will present and discuss recently discovered and unexplored features and facets of mosquito innate immune defenses against human pathogens.

Keywords:*Aedes, Anopheles, innate immunity, dengue, Plasmodium*

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Biochemical characterization of host-immune related molecules by infection of pathogenic or symbiotic bacteria into the hemimetabolous insect, *Riptortus pedestris*

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To investigate the biochemical basis of how insects recognize invading pathogenic microbes and how pathogen recognition signals are transferred to the down-stream factors and activate host innate immune responses, it is necessary to use large insects, from which larger amounts of hemolymph can be extracted and can be used to perform biochemical studies. We have recently determined molecular activation mechanisms of insect's Toll signaling pathway after purification of several pattern recognition proteins, serine proteases, serpins and AMPs. In this talk, we will discuss the biochemical characterization of these molecules, which are involved in the recognition of pathogenic microbes, in the amplification of recognition signal transduction and in the regulation of proteolytic cascade leading to the production of AMPs in insects. Recently, we started the studies to elucidate the differences of molecular innate immunities, which are induced by pathogenic microbes or symbiotic bacteria. The model system used in this study is Riptortus pedestris (bean bug), belonging to the family Alydidae, which is well known pest of leguminous crops in eastern Asia. As symbiotic bacteria, we selected Burkholderia species. Burkholderia symbiotic bacteria specifically localized in the lumen of the midgut crypts. Prevalence of the Burkholderia infection was consistently high, 95?100%, in natural populations of the alydid stinkbugs. We purified several bean-bug specific AMPs and have characterized several genes that specifically expressed genes after Burkholderia infection in the mid-gut. We will discuss the molecular mechanisms of how hosts distinguish pathogenic bacteria and symbiotic bacteria in order to defense their bodies.

Keywords:*host defense, pathogenic bacteria, symbiotic bacteria, Antimicrobial peptides (AMP)*

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Bacterial flagellin as an initiator of insect immune responses

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Flagellin is recognised by the immune system of the model lepidopteran insect Manduca sexta. Recombinant flagellins from Salmonella enterica Typhimurium and Photorhabdus luminescens TT01 are effective inducers of insect immune reactions in vivo, a single injection causing large increases in the mRNA levels of several different antimicrobial peptide (AMP) genes, together with smaller increases in the mRNA levels of two pattern recognition receptor proteins (PRRs). Flagellin also causes prophenoloxidase (PPO) activation in cell-free M. sexta hemolymph in vitro, indicating that in this case, flagellin must be detected by a secreted PRR. Different regions of the Salmonella flagellin protein molecule are differentially effective in causing AMP expression and PPO activation, indicating that there is more than one flagellin recognition mechanism in this insect.

Keywords:*Flagellin, antimicrobial peptide, phenoloxidase*

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Organisation of the cuticle as a physical barrier involves the C-type lectin Schlaff

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The insect cuticle is an apical extracellular matrix produced by polarised epithelial cells. Distinct distribution of chitin, proteins, catecholamines and lipids leads to a stratified structure that assumes the multiple functions of the cuticle as a physical barrier and as an exoskeleton. The apical plasma membrane of these cells plays a central role not only as a passive interface for cuticle material deposition but also as an active organising performer. Concomitantly, extracellular processes are deployed for terminal cuticle assembly. In the past few years others and we have identified several membrane associated and extracellular factors that are essential for cuticle organisation and function. One of these factors in the fruit fly Drosophila melanogaster is Schlaff, a secreted C-type lectin that we have shown to bind to mannose and N-acetylglucosamine. It localises to the basal site of the cuticle adjacent to the apical plasma membrane. Elimination of Schlaff function causes loosening of cuticle layers, and mutant animals suffer massive water loss. Intriguingly, removal of Schlaff within the imago head abrogates Ptilinum integrity, whereas hard cuticle parts appear to remain stable. We therefore hypothesise that Schlaff is needed to establish a physical barrier especially within soft cuticle (larval cuticle, Ptilinum) that antagonises the internal hydrostatic pressure. Since orthologuous sequences are present in other insects as well, this function is probably not confined to D. melanogaster. Further analyses of the Schlaff protein should reveal the molecular network of its activities.

Keywords:*Cuticle, Drosophila melanogaster, epidermis, hydrostatic pressure*

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S307TH02

Insect Immunology, Physiology and Neurobiology

Cuticular hydrocarbon biosynthesis

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Insects use hydrocarbons as cuticular waterproofing agents and as contact pheromones; however the last step of alkane biosynthesis from long chain fatty aldehydes has remained mysterious. Unlike cyanobacteria that make alkanes from aldehydes with a non-heme diiron decarbonylase, we show here that insects use a P450 enzyme of the CYP4G family to oxidatively produce terminal hydrocarbons from aldehydes. Oenocyte-directed RNAi knock-down of Drosophila CYP4G1 or NADPH-cytochrome P450 reductase both result in flies deficient in cuticular hydrocarbons and highly susceptible to desiccation or that die upon adult emergence. The heterologously expressed enzyme converts C18-trideuterated octadecanal to C17-trideuterated heptadecane, showing that the insect enzyme is an oxidative decarbonylase that catalyzes a novel cleavage of long chain aldehydes to alkanes and carbon dioxide. The highly conserved CYP4G enzymes of insects are a key evolutionary innovation that allowed their colonization of land.

Keywords:*cuticle, alkane, P450, oxidative decarbonylase, oenocytes, Drosophila melanogaster, Musca domestica*

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Cuticular hydrocarbons and behaviour in social insects

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Social insects (ants, termites and some bees and wasps) live in complex societies which are mainly coordinated through chemical communication. Especially cuticular hydrocarbons (CHC) seem to be involved in species identification, nestmate recognition, and status communication within colonies. The latter two are particular important as they ensure that altruistic behaviour is directed towards relatives and that colonies have few conflicts. The 'reproductive harmony' in social insects, expressed in the queen's reproductive monopoly, is often maintained via CHCs on the queen's cuticle that reveal her fertility to the non-reproductive workers. There has been fundamental progress in uncovering how CHCs function in insect societies. I will provide an update on this progress and present results for a termite species where the underlying molecular process of royalty signalling has been studied in detail. Using a combination of RNA interference, behavioural assays, endocrinological studies and chemical analyses we could identify a gene involved in the production of the queen's royalty scent in the lower termite Cryptotermes secundus. The expression of this gene, Neofem4, seems to be positively regulated by juvenile hormone. This makes Neofem4, a cytochrome P450 gene, a reliable indicator of the queen's fertility.

Keywords:*social insects, communication, cuticular hydrocarbons, termites*

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Molecular basis of color pattern formation in dragonflies

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Because dragonflies visually recognize conspecific and heterospecific individuals, their body color plays essential roles in their ecology and reproductive biology. Unlike most insects, some dragonflies change their body color dramatically upon sexual maturation. While many ecological and behavioral studies have been focused on this topic, molecular mechanisms underlying the color pattern formation have been poorly understood. Here we report our recent findings on molecular basis of color pattern formation in dragonflies. We analyzed pigments and genes that are involved in the different color patterns in dragonflies. Melanins, ommochromes, and pteridines represent the major pigment groups found in dragonflies. Notably, we found that sex-specific redox changes in ommochrome pigments cause the yellow-red color transition in some dragonflies, which unveils a previously unknown molecular mechanism underlying body color change in animals. We also identified several genes associated with different color patterns in dragonflies, which suggests that different molecular mechanisms are involved in the color pattern formation in different dragonfly lineages.

Keywords:*dragonfly, color pattern, pigment, melanin, ommochrome*

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Wing pigment patterns in the ladybird beetle, *Harmonia axyridis*

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Insect wing color patterns demonstrate a tremendous range of diversity and have evolved to fulfill various ecologically important functions such as intraspecific sexual signaling, mimesis, mimicry and warning against predators. The molecular mechanisms behind such pattern formation, however, remain unknown. To investigate the developmental mechanisms of color pattern formation, we chose the multicolored Asian ladybird beetle, Harmonia axyridis (Coleoptera), which has conspicuous and variable wing color patterns consisting of black and red pigments. Classical genetic studies revealed that the elytral color patterns are thought to be regulated by a single locus which gives multiple alleles, and that the color patterns are formed from the superimposition of any combinations of four major alleles. In addition, in any heterozygotes, the black-pigmented area invariably appears as dominant character at the overlapping black and red pigmentations where the color patterns of different alleles are superimposed. The mechanisms involved in wing color pattern formation remain unknown. As an initial step to understand the mechanisms, we examined histologically and biochemically the pigmentation of H. axyridis wing. Our observations suggest that carotenoids only localize in the region where no melanization occurs, and that melanin synthesis depends on dorsal cells, whereas carotenoids are accumulated in ventral cells. To elucidate the molecular mechanisms underlying the wing color patterns, we have established a technique for RNA interference (RNAi) in the ladybirds. We will report our progress in the expression and functional analyses of melanin-synthesis enzymes during wing development.

Keywords:*Harmonia axyridis, wing colour pattern formation, melanin, carotenoid, RNAi*

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S307TH06

Insect Immunology, Physiology and Neurobiology

Relationship of cuticular proteins and sclerotization with mechanical properties of beetle cuticle

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Insect cuticle is composed primarily of chitin, proteins, and catechol metabolites of tyrosine. Chitin fibers are embedded in a matrix of cuticular proteins, with additional stability provided through cross-links with catecholic molecules (sclerotization). The oxidized products of catechols also can polymerize to form the pigment melanin. The physical properties of cuticle can vary from hard and rigid to soft and flexible and from transparent to highly pigmented. We are using genomic information from the red flour beetle, Tribolium castaneum, for experiments aimed at understanding how variation in the cuticular protein composition and the metabolism involved in sclerotization and pigmentation affect cuticle properties using wings as model tissues. The forewings of beetles are modified to form heavily sclerotized elytra, which function as a protective covering, whereas the hindwings used for flight are formed from a flexible, membranous cuticle. Thus, the elytra and hindwings are two developmentally related structures that offer the possibility of examining both hard and flexible cuticles. We have analyzed the gene products present in elytra and hindwings by microarray and proteomics experiments. We observed preferential expression of RR-2 cuticular proteins in the elytra and RR-1 proteins in the hindwings. Elytra are also enriched in cuticular proteins with low sequence complexity. Two RR-2 cuticular proteins are highly abundant in elytral cuticle. RNAi experiments indicate that these two structural proteins are essential for morphogenesis of the elytra. Genes involved in catechol metabolism related to sclerotization and pigmentation also affect the mechanical properties of elytra. (Supported by NSF grant IOS0726425)

Keywords:*cuticular protein, sclerotization, tanning, structural protein, Tribolium castaneum*

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S307TH07

Insect Immunology, Physiology and Neurobiology

RNAi-based functional analysis of yellow-e in *Tribolium castaneum*

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Yellow protein (dopachrome conversion enzyme, DCE) is involved in the melanin biosynthetic pathway and significantly accelerates pigmentation and melanization reactions. Recent studies have suggested that yellow is a rapidly evolving gene family generating functionally diverse paralogs. However, the exact physiological functions of several yellow genes are still not understood. Injection of dsRNAs for Tcyellow-e (TcY-e) into last instar larvae had no effect on larval-pupal molting, and the resulting pupae developed normally. The pupal cuticle (e.g. setae, gin traps and urogomphi) showed normal tanning. Adult cuticle could also be viewed through the translucent pupal cuticle. Adult cuticle tanning (e.g. head, mandibles and legs) was initiated on schedule, indicating that TcY-e is not required for pupal or adult cuticle pigmentation in T. castaneum. The subsequent pupal-adult molt, however, was adversely affected. Although pupal cuticle apolysis and slippage were evident, some of the adults (~20%) were unable to shed their exuvium and died entrapped in their pupal cuticle. In addition, the resulting adults rapidly became completely desiccated. Failure of the pupal-adult molt and desiccation-induced mortality were prevented by maintaining dsRNA TcY-e-treated insects at 100% relative humidity (rh). When the humidity-rescued adults were subsequently moved from 100% rh to 50% rh, they quickly desiccated and died (wild-type insects thrive throughout development at 50% rh). Immunolocalization studies revealed the presence of TcY-e in the adult hard cuticle. These results suggest that TcY-e has a "critical waterproofing" function for cuticle rather than cuticle pigmentation. This work was supported by NRF (20110029585).

Keywords:*Tribolium castaneum, yellow, DCE, pigmentation, cuticle, exoskeleton*

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Laccase activation in ecdysis process

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Laccase is a multi-copper protein found in various organisms. In insects, laccase participates in cuticle formation by oxidizing phenolic compounds to generate highly reactive products which contribute to sclerotization and coloration of the cuticle. When synthesized beneath the old cuticle, the new cuticle is initially soft and colorless, but later becomes hardened and pigmented at specific time points during ecdysis. The completion of this process requires precise regulation of laccase activity in the newly synthesized cuticle. With the aim of elucidating the mechanisms underlying the sclerotization process, the molecular properties and expression pattern of laccase2 of the silkworm, Bombyx mori were studied. Transcripts of laccase2 (Bmlac2) are abundant before pupal ecdysis, and Bmlac2 protein accumulates at this stage of pupal cuticle formation. However, little laccase activity is detectable in the cuticle by in situ staining. Laccase activity becomes detectable only after pupal ecdysis. This observation suggests that laccase is present in the newly synthesized pupal cuticle as an inactive precursor that is activated after pupal ecdysis. An inactive form of Bmlac2 can be solubilized from the cuticles of newly ecdysed pupae by treatment of cuticles with α-chymotrypsin. The inactive Bmlac2 was purified to homogeneity as judged by SDS-PAGE. The purified laccase can be converted to active enzyme by treatment with proteases or buffered isopropanol, suggesting that the Bmlac2 precursor can be activated in vivo. The mechanisms involved in such putative activation remain unknown.

Keywords:*laccase*

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Functional analysis of chitin-binding proteins in the cuticle of *Tribolium castaneum*.

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An extensive bioinformatics search of the genome of the red flour beetle (T. castaneum) has identified genes encoding >50 proteins with the peritrophin A domain (ChtBD2), an ~65 amino acids-long sequence motif with its characteristically spaced set of six cysteines. Among these we have identified two families of genes encoding proteins that have one and three ChtBD2 repeats, which we have named cuticular proteins analogous to peritrophins, CPAP1 and CPAP3. The genome also encodes >100 proteins with the ~65 amino acids-long Rebers & Riddiford chitin-binding motif devoid of cysteines. We have also identified two other genes Knickkopf and Retroactive, which have been implicated in cuticle organization. All of these genes are uniquely expressed in cuticle forming tissues, but not in the gut. The individual members of the CPAP1 and CPA3 families differ in their expression patterns during development suggesting distinct functions. This was confirmed by RNA interference (RNAi) of specific members of these two families of genes. These studied have revealed functional specialization even among members of the same family. The phenotypes resulting from these RNAi experiments include molting arrest, reduction in chitin content, elytral, wing and joint abnormalities, loss of fecundity and defective egg hatch. Knickkopf and retroactive are involved in protecting and organizing chitin into laminae in the cuticle. The conservation of orthologs of these genes in different orders of insects suggest that they serve essential and non-redundant functions that affect cuticles in various parts of the insect anatomy and at distinct stages of development.

Keywords:*Insect cuticle, Peritrophin-A domain, chitin-binding, RNA interference,elytron, Knickkopf*

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Chitin synthesis inhibitors: Old molecules and new developments

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During the past decades, a larger number of chemically unrelated insecticidal compounds have been developed and commercialized that interfere with chitin biosynthesis, a process which is essential to growth and development of arthropods. Common symptoms after administration of these compounds are abortive molting and defects in egg hatching. Some of them are also effective in disrupting the barrier function of the peritrophic matrix. Chitin synthesis inhibitors are chemically diverse and include compounds such as pyrimidine-nucleoside peptides, benzoylureas, oxazolines and thiazolidinones. Pyrimidine-nucleoside peptides act as competitive inhibitors at the catalytic site of the chitin synthase, a membrane bound glycosyltransferase, which is considered the key enzyme of the chitin biosynthetic pathway. However, many of the commercialized acaricides and insecticides act at a post-catalytic step of chitin synthesis. Their mode of action has remained largely elusive. In a genomic and proteomic study we used Tribolium castaneum as a model for investigating the insecticidal effects of the benzoylurea compound, diflubenzuron. The results of this study recapitulate all of the different diflubenzuron-induced symptoms in a single model insect, which have been previously found in several different insect species, and further illustrate that diflubenzuron-treatment causes a wide range of effects at the molecular level. Recent progress in understanding the mode of action of the oxazoline compound, etoxazole, suggests that this chitin synthesis inhibitor targets directly the chitin synthase at a post-catalytic site, which could impair translocation of the nascent chitin chain across the membrane or oligomerization of single chitin synthase subunits.

Keywords:*diflubenzuron, chitin synthesis inhibitors, cuticle, etoxazole, molting, peritrophic matrix*

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Peptidergic signaling cascades in regulation of insect ecdysis

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Molting and ecdysis are distinctive and characteristic features of arthropod physiology and vital for growth and metamorphosis from juvenile to adult. These developmental and behavioral events are regulated by steroid and peptide hormones. Early in the molt, elevated ecdysteroid levels induce expression of genes important for scheduling tracheal dynamics and behavioral steps leading to ecdysis. These include the ecdysis triggering hormone (ETH) precursor gene in endocrine Inka cells and the ETH receptor gene (ETHR) expressed in sets of identified central neurons. Inka cells accumulate large amounts of ETH peptides during the molt, but acquire competence to release only shortly before ecdysis following expression of the late gene β-FTZ-F1. ETH release into the hemolymph initiates tracheal inflation, followed by pre-ecdysis, ecdysis, and post-ecdysis behaviors. These behaviors coincide with ETH activation of central peptidergic ensembles that release FMRFamides, eclosion hormone, kinins, CCAP, MIPs, and bursicon. Successive release of these peptides into the CNS and periphery activates central pattern generators that drive sequential behaviors. We are examining the roles played by these neurons in behavioral scheduling using genetics and physiological manipulations. Our findings offer insights into how peptidergic neural circuits regulating behaviors are assembled and activated.

Keywords:*steroid, peptide, kinin, bursicon, CCAP, eclosion hormone, MIP, behavior*

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The proctolin gene and actions of proctolin in the blood-feeding bug, *Rhodnius prolixus*

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Proctolin was the first insect neuropeptide to be sequenced and synthesized, and appears to be unique to arthropods. The proctolin gene has, however, only been found in the genomes of a few insect species, including Drosophila melanogaster, Tribolium castaneum, Tenebrio molitor, and Acyrthosiphon pisum. No proctolin gene has been identified in genomes of Apis mellifera, Aedes aegypti, Bombyx mori, Nasonia vitripennis, or Acromyrex echinatior and 3 other ant species, where proctolin and its receptor are now considered absent. A recent study on neuropeptides in Rhodnius prolixus failed to identify a proctolin gene, and it was suggested that R. prolixus also lacks proctolin (Ons et al., 2011, Insect Molecular Biology 20, 29-44). We have reinvestigated the possible absence, or in fact, presence of the pentapeptide proctolin in the Chagas' disease vector, R. prolixus. Here we report the cloning of the proctolin cDNA, and find the transcript to be expressed in the central nervous system (CNS) and some peripheral tissues. A single copy of proctolin is predicted to be processed from the prepropeptide. We have biochemically identified proctolin in CNS extracts and shown its distribution using proctolin-like immunoreactivity (PLI). PLI is found in processes on the salivary glands, male and female reproductive tissues, and heart and associated alary muscles. Proctolin-like immunoreactive bipolar neurons are found on the lateral margins of the common oviduct and bursa. Proctolin stimulates increases in contractions of anterior midgut and hindgut muscles, and increases heartbeat frequency. Proctolin is indeed present and biologically active in this medically-important bug.

Keywords:*myotropin, neuropeptide, heart, expression, gut*

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The brown planthopper, *Nilaparvata lugens* is a new tool for studying the diversity of neuropeptide-signaling in insects.

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The brown planthopper, Nilaparvata lugens (Stal), is one of the most problematic insect pests of rice in Asia. The understanding of neuropeptide-signaling in N. lugens may enable the design of a new insecticide that resolves the problem of resistance to various insecticides since the insecticide targeting neuropeptide-related genes has not been developed so far. We have identified neuropeptide genes and G protein-coupled receptors (GPCRs) for neuropeptides comprehensively by combining homology search in the genome sequence and in RNA-seq data with cDNA cloning. N. lugens has the highest number of neuropeptide precursor genes compared to other insects with fully sequenced genomes. The genes encoding prothoracicotropic hormone (PTTH), inotocin, and their receptors were identified in N. lugens although these genes had not been identified in the other hemimetabolous insects. An orphan GPCR homologous to vertebrate parathyroid hormone receptor was identified in N. lugens. This gene has orthologs in several insect species but the function remains to be known. RNA interference (RNAi) of several neuropeptide and GPCR genes, e.g., PTTH and adipokinetic hormone receptor (AKHR), resulted in the disruption of development although the knockdown of these genes have not been reported to cause mortality in other insects. This result suggests that the physiological importance or function of structurally related neuropeptides is diverse among insects.

Keywords:*neuropeptide, G protein-coupled receptor, RNAi, Nilaparvata lugens*

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Diuretic and anti-diuretic strategies in the blood-gorging bug, *Rhodnius prolixus*

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Serotonin is a diuretic hormone in R. prolixus, but there is also ample evidence for the presence of a peptidergic diuretic hormone. The identity of this peptide has remained elusive; but recently, using molecular techniques and mass spectrometry, we have sequenced the peptidergic diuretic hormone and find it to be a member of the CRF-related family of insect neuropeptides. Rhopr-CRF/DH has potent biological activity on anterior midgut and Malpighian tubules, and works synergistically with serotonin during rapid post-feeding diuresis in R. prolixus. Cardioacceleratory peptide 2b (CAP2b) belongs to a family of neuropeptides (now referred to as CAPA peptides) that can serve diverse roles in the regulation of water and salt homeostasis in insects. These peptides stimulate fluid secretion by Malpighian tubules in dipterans, thus serving a diuretic function, whereas they have an anti-diuretic role in R. prolixus and some other hemipterans. We have identified two paralog genes which encode Rhopr-CAPA peptides, and also identified the G protein-coupled receptor that is activated by one of these peptides, Rhopr-CAPA-2. This anti-diuretic peptide and cognate receptor are important mediators in the cessation of rapid post-feeding diuresis in R. prolixus, inhibiting serotonin-stimulated secretion by Malpighian tubules, but not, apparently, Rhopr-CRF/DH-stimulated secretion. Rhopr-CAPA-2 appears to prevent the synergism between the diuretic hormones serotonin and Rhopr-CRF/DH. The interplay between diuretic and antidiuretic hormones in R. prolixus results in a remarkable diuresis; a diuresis that has evolved to eliminate excess water and salts from a massive blood meal, whilst maintaining homeostasis within the haemolymph.

Keywords:*Neuropeptides, Serotonin, Diuresis*

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Evolution of family-B GPCRs in insects and its function in *Tribolium castaneum*

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Family-B of G protein-coupled receptors (GPCRs) is associated with the regulation of circadian rhymicity, water and ion balance, lifespan and reproduction and other biological processes. However, it was less focused in the past years. Taking advantage of the recently sequenced insect genomes, we performed a genome-wide analysis of family-B GPCRs through 6 insects by using known GPCRs as references. Totally, we have identified 64 family-B GPCRs, 20 from Tribolium castaneum, 11 from Apis mellifera, 9 from Bombyx mori, 9 from Acyrthosiphon pisum, 14 from Anopheles gambiae and 1 novel member from Drosophila melanogaster. The result suggests that they are of ancient origin and have evolved through gene duplication/lost events. Phylogenetic and gene structure analysis have revealed 3 primary kinds of evolutionary patterns of family-B GPCRs during its evolution. Furthermore, the function and signal transductions of family-B GPCRs have been characterized in Tribolium castaneum by RNAi and high throughput methods, this work will provide a framework of information on family-B GPCRs of Tribolium and other non-drosophiline insect. It will significantly promote the functional analysis of family-B members.

Keywords:*GPCR, Family-B, Mth-like, Evolution, Tribolium castaneum*

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Bursicon homodimers induce immune gene expression

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Bursicon is a heterodimer neuropeptide composed of two cystine knot proteins, bursicon α (burs α) and bursicon β (burs β), that elicits cuticle tanning (melanization and sclerotization) through the Drosophila leucine-rich repeats containing G protein-coupled receptor 2 (DLGR2). Recent studies show that both bursicon subunits also form homodimers. However, biological functions of the homodimers remain unknown. Using recombinant bursicon protein, we have shown in Drosophila melanogaster that both bursicon homodimers induced expression of genes encoding antimicrobial peptides (AMPs) in neck-ligated adults following recombinant homodimer injection and in larvae fat body after incubation with recombinant homodimers. Up-regulation of AMP genes by the homodimers was accompanied by reduced bacterial populations in fly assay preparations. The induction of AMP expression is via activation of the NF-κB transcription factor Relish in the immune deficiency (Imd) pathway. These results reveal a mechanism of CNS-regulated prophylactic innate immunity during molting via induced expression of AMPs.

Keywords:*Bursicon, Homodimer, Antimicrobial peptide, Relish, Prophylatic immunity*

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Molecular physiology of tick salivation

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Ticks are obligatory ectoparasites causing global animal and human health problems. Ticks transmit a wide variety of pathogens, while they also directly damage the host. Nervous control of the salivary gland in tick is a critical step in blood feeding, since it is required for activation of salivary secretion and osmoregulation during blood feeding. While previous studies identified dopamine and arachidonic acid as compounds that activate salivary secretion, our recent finding indicates presence of more complex mechanisms for control of the salivary glands. We identified at least two neuropeptides, myoinhibitory peptide and SIFamide, innervating the salivary glands in blacklegged ticks (Ixodes scapularis Say) which transmits the most important tick-born disease Lyme disease. We hypothesize that these neuropeptides control dopaminergic cells located on the base of acini in the salivary glands. The fundamental knowledge obtained from this research is eventually expected to lead to the design of compounds and vaccines to prevent disease transmissions by ticks.

Keywords:*neuropeptide, dopamine, biogenic amine*

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S309F01

Insect Immunology, Physiology and Neurobiology S309

Mechanisms that underlie learning and memory of complex floral odors by honey bees and moths

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Flower odors are highly variable combinations of several volatile components. No two flowers smell exactly alike.. Honey bees must therefore establish if a newly encountered flower is similar enough to a previous rewarded one, turning foraging decisions into a fine tuned generalization-discrimination problem. Sensory and neural systems must provide mechanisms for very precise odor recognition, allowing for perceptual stability. We hypothesize that experience with odors tunes sensory processing and thereby improves odor recognition and classification of newly encountered flowers. To test this hypothesis, we have designed artificial floral blends that mimic the components, proportions and variability of two natural varieties of snapdragon flowers. All designed blends share the same components. But they can be differentiated based on the relative concentration of the components, which were more similar within than between cultivars. Bees were differentially conditioned using examples of both cultivars. After training we tested the ability to recognize a new example from each cultivar. The duration of PER was lower and the latency longer when an example of the non-rewarded cultivar was offered to the trained bee. Odor induced activity patterns were measured in Projection Neurons of the Antennal Lobe by calcium imaging . Consistent with behavior, results suggest that the neural network in the AL is tuned via differential conditioning to decorrelate mixtures representing different floral varieties. Experience-dependent plasticity at the level of the Antennal Lobe may help animals categorize a newly flower as belonging to a class related to reinforcement.

Keywords:*floral odors, mixtures, variability, honey bee*

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S309F03

Insect Immunology, Physiology and Neurobiology

Representation of behaviorally relevant odor information in the *Drosophila* brain

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Insects must decide how to react to detected olfactory stimuli based on complex input. The fruit fly Drosophila melanogaster evaluates food sources based on such cues. We used a bioassay to establish the innate valence of 110 odorants in the fruit fly. We analyze neuronal activation patterns evoked by attractive and aversive odorants and show that even though the identity of odorants is coded by the set of activated receptors, the main representation of odorant valence is formed at the output level of the antennal lobe. The topographic clustering within the antennal lobe of valence-specific output neurons resembles a corresponding domain in the olfactory bulb of mice. The basal anatomical structure of the olfactory circuit between insects and vertebrates is known to be similar; our study suggests that the representation of odorant valence is as well.

Keywords:*Drosophila, olfaction, valence, antennal lobe*

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S309F04

Insect Immunology, Physiology and Neurobiology

From sensory receptors to functional neural networks: Constructing and deconstructing olfactory circuits in *Drosophila*

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Insects detect volatile chemicals in their environment via olfactory receptors expressed on the surface of olfactory receptor neurons (ORNs). There are three receptor gene families that were shown to function in Drosophila olfaction: odorant receptors (ORs), ionotropic glutamate receptors (IRs) and gustatory receptors (GRs). In the Drosophila olfactory system, 1200-1300 ORNs, housed in clusters of 1-4 cells within olfactory sensilla on the antenna and the maxillary palps (MP), are specified to generate 50 different ORN classes. Each ORN class is defined by the exclusive expression of generally one olfactory receptor gene from a large genomic repertoire and their class-specific connectivity in the brain. How ORNs are specified and choose to express a single olfactory receptor gene remains an unanswered question. As the ORNs in the same sensilla develop from the same olfactory precursor cells, the sensory receptor potential of the pluripotent precursor cells is a critical determinant of the olfactory receptor expression by ORNs in each sensillum. Sequential restriction of sensory receptor potential of precursor cells can be achieved spatio-temporally during development, determining the specific receptor subsets to be expressed in specific sensilla. We have been investigating the role of transcription factors that regulate specification and olfactory receptor expression by ORN classes at the level of the precursor cells and epigenetic networks that function at the level of individual ORNs. The results from these studies will be discussed.

Keywords:*olfactory receptor expression, ORN specification*

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S309F05

Insect Immunology, Physiology and Neurobiology

Introceptive sugar sensing in *Drosophila*

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In past 30 years, there has been a dramatic increase in obesity around the world. This growing obesity pandemic is at least in part ascribed to poor eating habit. However, because eating behavior is influenced by multiple factors such as nutritional needs and food palatability, it has been difficult to investigate how this behavior is regulated. Peripheral chemosensory neurons such as sugar receptor neurons endow animals with the ability to detect palatable food. Additional mechanisms would exist for the detection of foods that meet nutrient needs. Indeed, my laboratory showed that the Drosophila mutants- GR5a;GR64a and pox-neuro mutants- that are insensitive to the taste of sugar still developed a preference for a sugar solution based on its nutritional value after prolonged periods of food deprivation. These findings suggest that there exists a taste-independent, internal sugar sensor that detects its caloric content. Using this two-choice preference assay (D-glucose versus non-metabolizable L-glucose), we carried out a small-scale screen for an internal sugar sensor and identified a mutation in a Na+/Glucose co-transporter, dSGLT11, that was completely insensitive to the caloric content of sugar, but rather responded only to the concentration of sugar- the "sweetness". Intriguingly, tissue specific knock-down of dSGLT11 specifically in the nervous system caused the dSGLT11 mutant phenotype. Consistent with this, dSGLT11 is expressed in few regions of the brain including the antennal lobe, the subesophageal ganglion, and the central complex. This restricted expression pattern postulated a hypothesis that dSGLT11 functions as a glucose sensor instead of a mere glucose transporter.

Keywords:*Endo-sensor, Taste-indepedent food choice behavior, Na+, Glucose co-transporter, Nutritional value*

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S309F06

Insect Immunology, Physiology and Neurobiology

The speed of smell: Odor-object segregation within milliseconds

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Segregating objects from background has been investigated in depth in vision and audition (figure-ground segregation, cocktail party effect) but has hardly been investigated in olfaction. Natural olfactory stimuli fluctuate at fast timescales, and it is not known whether and, if so, how animals use this temporal information to segregate concurrent odor-objects from independent odor sources. We addressed this issue by combining behavioral and physiological experiments in honeybees. We asked whether honeybees can use short temporal differences between two components of a binary odorant mixture to extract information about its components. We first trained honeybees to respond to an odorant A by pairing A with a sugar reward. Then, we tested memory retrieval with a mixture of A and a novel odorant B. We found that a 6 ms asynchrony in the onset of A and B is sufficient to enhance the salience of the component odor information, and that it is not necessary that the component in question was presented alone at any time during the stimulus. We then searched for a neural mechanism of odor-object segregation and asked whether olfactory projection neurons in the antennal lobe are influenced by short time delays in odor component onsets in mixtures. Using in vivo calcium imaging we found that the processing of temporally incoherent mixtures with 5 to 600 ms odor-onset delays between the components involved more inhibitory interactions than the processing of coherent mixtures.

Keywords:*olfaction, calcium imaging, odor mixtures*

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Plastic control of olfactory pheromone responses in *Drosophila*

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How does an animal extract salient information from its complex and variable environment? In fruit fly, Drosophila melanogaster, cis-vaccenyl acetate (cVA), a product of the male ejaculatory bulb, works as a courtship inhibitory pheromone. Previously we found that a male was capable to detect tiny amount of cVA and decreased his courtship motivation. However, it was unclear how the male could detect such a tiny amount of exogenous cVA without disruption by his own self-produced cVA. In this study, we predicted that olfactory sensitivities were constantly adjusted by the pheromonal environment to allow a male to ignore his own smell and focus on novel stimuli, and performed physiological analysis using a neural activity marker and behavioral analysis coupled by genetic manipulation using RNAi. First we found that prolonged exposure to purified cVA caused an enhanced level of subsequent courtship activity. This indicated the presence of homeostatic modulation, in which artificial activation of courtship inhibitory pathway increased basal activities and/or sensitivities of the counterpart courtship stimulatory pathway. Blocking the cholinergic excitatory input to the presynaptic terminals of the sensory neurons completely diminished this experience-dependent sensory modulation, suggesting that acetylcholine played a critical role for lateral communication between two groups of pheromone pathways and maintenance of signal equilibrium between them for precise pheromone coding.

Keywords:*olfaction, pheromone, plasticity, courtship, acetylcholine*

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S309F08

Insect Immunology, Physiology and Neurobiology

Specialized noses for specialized lifestyles

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The olfactory system directly interfaces with the environment, thus, changes in the environment, or a change in an animal's habits, as e.g. specialization, would presumably also lead to changes in the olfactory system. Comparative studies on specialized animals, with known generalist ancestors, could hence be a way of revealing general processes shaping olfactory systems, as well as highlighting importance and function of specific chemosensory genes. In this presentation I will outline current work in our laboratory dealing with olfactory adaptations at the molecular, physiological, morphological and behavioral level in a set of highly specialized drosophilid flies.

Keywords:*Drosophila Olfaction Odorant receptors*

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S309F09

Insect Immunology, Physiology and Neurobiology

Evolution of the insect chemoreceptor superfamily

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The insect chemoreceptor superfamily are ligand-gated ion channels that serve as gustatory and olfactory receptors. It is divided into two families phylogenetically. The ancestral GR family of largely gustatory receptors can now be traced back through animal evolution, including Mollusca, Annelida, and Nematoda, as well as basal deuterostomes, to Cnidaria and Placozoa, but was lost in Chordata. The OR family of odorant receptors clearly evolved from within the GRs, apparently as a specialization for terrestriality in insects, because the other three arthropods with genome sequence (Daphnia, tick, and centipede) do not have ORs. Within the insects, the repertoire size of ORs and GRs is generally concordant with the known complexity of chemical ecology of the insect, ranging from small in human body louse to large in ants. Recent results include a reduction of the OR and GR families in the tsetse fly, reduction of GRs but expansion of ORs in the hessian fly, and modest expansion of these families in a termite, which instead has a large expansion of the unrelated IR (ionotropic receptor) family of candidate gustatory and olfactory receptors.

Keywords:*odorant receptor, gustatory receptor, ionotropic receptor*

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Optophysiological approaches to olfactory signal transduction and odor processing in larval *Drosophila*

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Olfactory signal transduction and odor processing in insects is subject to intense research. In Drosophila, olfactory signal transduction depends on the co-expression of a specific odorant receptor and the non-specific Or83b co-receptor (Orco). Recent studies suggested the generation of a receptor potential via a second messenger independent pathway [1]. However, an additional involvement of the second messenger cAMP has been proposed [2]. We use optogenetic techniques in combination with behavioral analysis to investigate molecular components for olfactory signal transduction in Drosophila larvae. Through the expression of a light sensitive adenylate cyclase (Pacα) [3] in OSNs cAMP levels can be artificially increased through light stimulation. As has been shown previously [4], we observed that a positive chemotaxis can be induced by light stimulation. In the absence of endogenous Orco, the Pacα-induced behavioral response is largely abolished, indicating that cAMP affects olfactory signaling and that Orco is a target of cAMP. On the contrary, when larval OSNs were activated using channelrhodopsin-2 a light-induced positive taxis behavior was observed with or without Orco. Based on these results we use calcium imaging to visualize odor-induced and light-induced activity of larval olfactory neurons and investigate possible functional implications of cAMP within the larval olfactory signal transduction system. [1] Sato et al., 2008. Nature 452:1002-6. [2] Wicher et al., 2008. Nature 452:1007?1011. [3] Schroeder-Lang et al., 2007. Nat Methods 1:39?42. [4] Bellmann et al., 2010. Front Behav Neurosci 2:4-27.

Keywords:*Drosophila, optogenetics, optical calcium imaging, olfaction*

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A microRNA-based screen for novel genes involved in olfactory physiology

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Insects use their olfactory systems to find food and mates and to avoid danger. The behavioral responses to these important external cues are guided by subpopulations of olfactory sensory neurons (OSNs) that express receptors that recognize behaviorally relevant volatile odorants. Although the role of the odorant receptors (ORs) in determining a given olfactory neuron's odor response profile is well-established, the more subtle mechanisms that may lead to different outcomes depending on environmental and behavioral context are less well-known. We are, therefore, interested in identifying novel genes expressed in olfactory neurons that modulate olfactory physiology. To do so, we have generated a library of >150 fly stocks that permit the tissue-specific overexpression of Drosophila miRNAs. MicroRNAs are single-stranded RNAs of approximately 22 nucleotides in length that play an important role in regulating gene expression by binding sequence-specifically to their target mRNAs and inducing their degradation. By crossing these UAS-miRNA lines to the Orco-GAL4 line, which is highly expressed in most olfactory neurons, we have used single sensillum electrophysiology to identify miRNAs that modulate the odor-evoked responses of OSNs in basiconic sensilla. Since the available methods for miRNA target prediction are prone to generating false positives, we are currently carrying out a smaller secondary screen using UAS-RNAi lines for predicted targets to identify the specific target mRNA(s) that are being affected in the positive hits from our primary screen.

Keywords:*Drosophila, olfaction, miRNA*

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Structural and functional differences in the antennae of worker honey bees of *Apis mellifera* and *Apis cerana*

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Olfactory discrimination, perception, and efficient orientation abilities to food resources play important roles for most insects in survival. The complexity of olfactory systems in a given species may depend on its feeding ecology and chemosensory abilities involved in inter and intra-specific recognition in their environment. The honey bee is a highly social insect and works together by a division of labor. To regulate the complex social interactions, honeybees have evolved an intricate system of special olfactory organs. To understand the differences of olfactory functional mechanisms in honeybee antenna, the structural and functional variations of worker bee antennae of two honey bee species, Apis mellifera and Apis cerana were compared using electron microscopic analysis, electrophysiological measurement, and proboscis extension reflexes (PER). Our present study showed that putative olfactory sensilla (placodea, trichodea, basiconica) were more significantly abundant on the A.cerana antenna than A.mellifera. Similarly, olfactory responses to various floral volatile compounds were higher in A.cerana than A.mellifera. In addition, PER responses were slightly different in two species. These findings may extend our understandings of the evolution of honey bee olfactory systems and behavioral traits to various ecological and biological signals between two closely related honey bee species.

Keywords:*Honey bee, Olfaction*

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S401M01

Development and Reproduction

Neurohormonal control of desert locust (*Schistocerca gregaria*) reproductive physiology

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The desert locust (Schistocerca gregaria) is a feared pest insect. Swarms consisting of billions of individuals can seriously damage the crop production in large areas of the world. The reproductive process of these animals may constitute an interesting target for pest control strategies and this fact stimulates fundamental research of locust reproduction. We recently produced an EST database derived from desert locust central nervous system (Badisco et al., 2011). Homology searches resulted in the identification of multiple transcripts encoding neuropeptides and lipophilic hormone biosynthesis enzymes that may be candidate key players in desert locust reproductive physiology. The availability of this novel transcript sequence information meant a great step forward in the desert locust reproduction research and allowed us to confirm the involvement of some neuropeptides in the process of vitellogenesis. Not only the production of mature gametes, but also the acquirement of the necessary nutritional energy is an essential part of the reproductive process. Some neurohormones may therefore indirectly control the locust's reproductive physiology by regulating feeding mechanisms and/or by acting as signal molecules that sense whether the required nutritional conditions are fulfilled to proceed to the reproductive processes. We recently studied the possible role in desert locust reproductive physiology of insulin-related peptide, neuroparsins, ovary maturating parsin/diuretic hormone, neuropeptide-like F and juvenile hormone biosynthesis enzymes.

Keywords:*desert locust, neurohormone, vitellogenesis*

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The distribution and physiological effects of neuropeptides controlling reproductive tissues in the kissing bug, *Rhodnius prolixus*

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Coordination of muscle contractions of the reproductive system in both male and female insects is critical for successful mating and the eventual production of viable eggs. This coordination involves a variety of neurons and their neuroactive chemicals. We have described these events in detail in the locust, Locusta migratoria, and shown that male and female reproductive tissues are under central neuronal control, with the neural substrate expressing an array of peptide and amine phenotypes. Central pattern generators (CPGs) are involved in oviposition, and the CPGs that control digging of the ovipositional hole, egg retention in the lateral oviducts and sperm release are synchronized. More recently, we have been examining the male and female reproductive systems of the medically-important bug, Rhodnius prolixus. Here again, these systems are under neuronal control, the neurons of which utilize a variety of neuropeptides to influence muscle contraction which again leads to successful mating and egg-laying. These peptides include proctolin, FGLamide/ASTs, FMRFamide-like peptides and myoinhibiting peptides (MIPs). The MIPs are believed to be the ancestral ligands for the Drosophila sex peptide receptor. The MIP precursor in R. prolixus encodes MIPs that have an unusual W(X7)Wamide motif. MIP-like immunoreactivity is found within neurons projecting to the female and male reproductive systems, and these peptides, along with others, influence muscle activity.

Keywords:*reproduction, MIPs, proctolin, allatostatins, myosuppresin, FMRFamide-like peptides*

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S401M03

Development and Reproduction

RNAi Technology reveals the roles of G-protein coupled receptors in moth reproductive behavior

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Sex-pheromone production in the night flying female moth, Helicoverpa armigera is under neuroendocrine control due to Pheromone Biosynthesis-Activating Neuropeptide (PBAN) at which time calling females attract conspecific males. On reaching the females, males display their hair-pencil complexes that contain fatty acid derived "pheromonal" compounds, structurally similar to female sex-pheromones. The role of pheromone biosynthesis activating neuropeptide (PBAN) in the regulation of female sex-pheromone biosynthesis is well elucidated, but its role in males remained a mystery for over two decades, since its discovery from both male and female central nervous systems. Through RNAi-mediated gene-silencing of the G-protein coupled PBAN-receptor gene, we revealed the functionality of the PBAN-receptor and the key components that are up-regulated by PBAN in male hair-pencil complexes. After mating, females undergo drastic behavioral and physiological changes including the termination of sex-pheromone biosynthesis and an increase in oviposition behavior. Synthetic Drosophila melanogaster Sex-Peptide (DrmSP), which is responsible for terminating receptivity in female flies, can also terminate PBAN-stimulated sex-pheromone production; suppress calling behavior; and down-regulate the PBAN-receptor transcript levels. We identified a G-protein coupled receptor for Sex-Peptide in H. armigera (HeaSP-R) and a differential expression study revealed up-regulation of HeaSP-R transcript levels in female neural tissues and pheromone glands after mating. RNAi-mediated gene-silencing of the HeaSP-R expression by 50-60% prevented DrmSP-suppression of pheromone production and calling behavior. Moreover, mated, silenced females failed to increase their oviposition rates and their behavior did not differ from that of virgin females thereby implicating Sex-Peptide in the post-mating behavioral changes of this moth.

Keywords:*PBAN, Sex Peptide, Sex Pheromone*

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The potential for disrupting pupal diapause in *Heliothis/Helicoverpa* with diapause hormone

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Diapause hormone (DH), a 24-amino acid neuropeptide in the pyrokinin family, is best known for its action in inducing diapause in embryos of the silkmoth, Bombyx mori. But, interestingly, this same hormone can be used to break the pupal diapause in members of the Heliothis/Helicoverpa complex of pest species. Based on the structure of DH, we designed several agonists that are much more active than DH in breaking diapause. One of the agonists is 50x more potent than DH itself. One such agonist also prevents the entry into diapause when administered to larvae that have been environmentally programmed by short day length to enter diapause. We also used a unique antagonist development strategy of incorporating a dihydroimidazole ("Jones") trans-Proline mimetic motif into one of our DH agonists, thereby converting the agonist into a DH antagonist that blocks the termination of diapause. Thus, these new agents, which can be used to prevent, terminate, or block the termination of diapause, suggest possibilities for compromising the overwintering success of these pests insects.

Keywords:*Diapause, neuropeptides, diapause hormone*

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Neuroparsin

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The pars intercerebralis is the major center of neuroendocrine cells in the insect brain, which are grouped into the pars intermedia and the bilateral pars lateralis. Two cell types in the pars intermedia are stained using the classical neurosecretory stain paraldehyde fuchsin, one of these produce the insect insulin related peptides, and the other a hormone which is only known from arthropods, neuroparsin. Both cell types are almost ubiquitously present in insects, but the neuroparsin cells are absent from Drosophila melanogaster and other closely related Drosophila species. Given their strong affinity for paraldehyde fuchsin, the neuroparsin neuroendocrine cells were probably the first insect endocrine cells identified. Although we know very little about the function of this neurohormone, neuroparsin is a fascinating subject for conjecture and speculation as will be discussed.

Keywords:*Neuroparsin*

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Possible ETH role in regulation of juvenile hormone production in Bombyx

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The silkworm Bombyx mori is a promising model species for functional analysis of neuroendocrine interactions controlling development and behavior. We used various molecular, biochemical and physiological approaches to study functions of ecdysis triggering hormones (ETH) from endocrine Inka cells and its ETH receptors (ETHR). Careful examination of ETHR expression using in situ hybridization and qRT-PCR revealed appearance of this receptor in multiple peptidergic and aminergic neurons of the CNS and in endocrine cells of the corpora allata (CA) prior to each ecdysis. Particularly strong ETHR expression was detected in endocrine cells of the CA several hours after each ecdysteroid peak in pharate larvae, pupae and adults. Increased levels of juvenile hormones (JH) following each ecdysis coincide with ETH release and suggest its possible direct action on the CA. Indeed, our preliminary experiments indicate that ETH elicits in vivo JH biosynthesis in pharate larvae. To further analyze the role of ETH-ETHR signaling in JH biosynthesis, we identified a CA-specific promoter for an enzyme aceto-acetyl CoA thiolase (AACT) using the baculovirus expression system. The AACT promoter with GCaMP and ETHR were subcloned into the piggyBac vector for production of transgenic animals. Resulting transgenic lines have been used for Calcium imaging and physiological experiments in vivo and in vitro. These experiments revealed unexpected neuroendocrine pathways between specific central neurons, Inka cells and corpora allata. Supported by Slovak grant agencies APVV (51-039105), VEGA (2-6090-26) and NIH (GM 67310).

Keywords:*ETH receptor, corpora allata, Bombyx mori, juvenile hormones*

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Juvenile hormone titer and allatoregulating neuropeptides in wood-feeding cockroaches and primitive termites

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Termites are eusocial insects with differentiated castes. They have two developmental systems, the linear pathway system or the dual-line system, and juvenile hormone (JH) plays a central role in caste regulation. Several studies have shown that JH titers are elevated in the course of soldier development. The synthesis of JH in the corpora allata of insects is regulated by allatostatins (AST) and allatotropins. Allatostatins of the FGLamide type (A-type) occur in many insect orders, but are known to inhibit JH biosynthesis only in cockroaches, crickets, and termites. The gene for the AST-A preprohormone has been identified in several cockroaches, but only in one termite species, Reticulitermes flavipes. We identified the gene for the prepro-AST peptides in the only genus of the basal termite family Mastotermitidae, the Darwin termite, Mastotermes darwiniensis. In addition, the expression of the gene in brain and gut of all developmental stages and castes is demonstrated. The gene codes for 14 individual peptides and its sequence is most similar to that of the xylophagous woodroach, Cryptocercus darwini. There is a strong negative correlation between expression of the AST-A gene in the brain and the haemolymph JH III-titer in different developmental stages of the fertile and the sterile line of M. darwiniensis (measured by HPLC-MS). Knock-down of the AST-A gene by RNA interference, however, did not affect the JH titer of female and male workers. Our results confirm wood-feeding cockroaches as direct ancestors of termites, as well as the FGLamides as true allatostatins in both insect groups.

Keywords:*allatostatin, juvenile hormone, termite, cockroach*

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RNAi of the allatotropin peptide precursors and allatotropin receptor highlighting their physiological role in insects: Focus on the red flour beetle, *Tribolium castaneum*

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In Tribolium we recently characterized the allatotropin GPCR receptor. RNAi knockdown started in the last larval stage of either receptor (Tc atr) or all three allatotropin-like peptide precursors. No developmental effects were observed. The limited number abnormal developmental phenotypes were not different from control gfp RNAi larvae. Compared to females we counted a significant lower number of emerging RNAi surviving receptor knockdown males. Being validated by an RNAi knockdown of all the allatotropin precursor isoforms simultaneously, this points to an important role of JH in developing and sexually maturing males of Tribolium. Importantly, these Tc atr RNAi males display a reduced reproductive capacity when mated with wild-type unmated females. Tc atr receptor transcript levels are surprisingly high in male accessory glands and predict a role for JH in male reproduction and accessory glands development in Tribolium males. Accordingly, the RAG glands have significant smaller sizes in Tc atr RNAi males compared to controls as is the case in JHAMT RNAi Tribolium males (Parthasarathy et al., 2009). Given the absence of this phenotypic effect on accessory glands in Trica-atl RNAi males, the receptor seems to be the limiting factor in stimulating JH synthesis/release by the male accessory glands. We can expect the high but incomplete knockdown of the allatotropin precursor mRNA's still

Keywords:*Accesory glands, JH, GPCR, RNAi*

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Epigenetic control of locust phase polymorphism

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The morphological, physiological and behavioral differences between solitarious and gregarious desert locusts are so pronounced that one could easily mistake the two phases as belonging to different species.. A number of phase-specific features are hormonally controlled. Juvenile hormone promotes several solitarious features, the green cuticular colour being the most obvious one. The neuropeptide corazonin elicits the dark cuticular colour in the gregarious phase in addition to particular gregarious behavioral characteristics. However, endocrine factors may not be the primary cause in phase determination. Our observation that longevity gets imprinted very early in life and that it cannot be changed thereafter, made us consider the possibility that epigenetic control of gene expression might be the primary phase-determining mechanism. Imprinting is likely to involve DNA methylation and histone modification. Analysis of a Schistocerca EST database of nervous tissue identified the presence of several genes that may be involved in epigenetic regulation, including two DNA methyltransferases (Dnmts). Dnmt1 and Dnmt2 are phase-specifically expressed in certain tissues. In the metathoracic ganglion, important for serotonin mediated sensing of mechanical stimuli, the level of Dnmt1 and 2 expression is affected by crowding. Pilot methylome sequencing data indicate that DNA methylation in the desert locust goes up to 1.4% genomic cytosine methylation in the brain and metathoracic ganglion. This is the highest level observed in insects so far. So far, there is no evidence for a Dnmt3, a de novo DNA methyltransferase in locusts, which makes the desert locust a prime epigenetic model to study DNA methylation.

Keywords:*locust phase polymorphism, epigenetics, DNA methylation*

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S401M10

Development and Reproduction

Functions of sNPF/CCAP autocrine metabolostat regulating feeding, behavior and reproduction in *Periplaneta americana*

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Insects must adjust their physiological and behavioral conditions, according to the availability of food, allocating nutrients in their reservoir to most relevant function. Namely, starvation requires to shut down digestive activities, protein synthesis, mitosis and reproduction and to mobilize stored reservoir and stimulate autophagy and apoptosis. The refeeding reverses this process. The brain-midgut axis employs a variety of neurotransmitters, neuropeptides and growth factors in integrating physiological, metabolic, behavioral and reproductive functions in the American cockroach, Periplaneta americana, notably as we have shown (Sakai et al. 2004; Endocrinology 145,5671 and 2006 Peptides 27, 2157). CCAP from the brain and the midgut epithelium, interact and regulate digestive activities in the midgut.These brain-midgut interplay is affected by circadian oscillation, since the removal of pars intercerebralis upregulates the number of CCAP- and allatostatin-ir paraneurons, inducing hypertrophy only when the animal is placed in constant darkness (Matsui et al. 2009 Physiology and Behavior 96, 548 and unpublished data). sNPF production is enhanced under starvation both in the brain and midgut paraneurons. It is down-regulated by refeeding and CCAP injection, while CCAP is down regulated by sNPF but upregulated by refeeding.Since sNPF-ir and CCAP-ir were colocalized both in some neurons and paraneurons, this system functions as an autocrine system that regulates global physioology, behavior, metabolism and reproduction, since the injection of sNPF and starvation upregulate while the injection of CCAP and refeeding down-regulate locomotory activities. In Leptinotarsa dessimlineata, starvation suppresses the CA activity thus reproduction. Starvation often induces oosorption. Here the neuropeptide mechanism will be elucidated.

Keywords:*sNPF, CCAP, allatostatin, JH,*

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S401M11

Development and Reproduction

Behaviour-modifying peptides and peptidases of male accessory glands

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Mating elicits various physiological and behavioural responses in post-mated female insects (e.g. male rejection and elevated ovulation rate). We have studied the effect of mating on wakeful activity and sleep in female drosophilids. In D. melanogaster, copulation promotes daytime wakefulness in the post-mated female; a change of behavior compatible with increased foraging and egg?laying activity. Extending this study to other Drosophila species suggests that the effects of mating on adult female wakefulness are species dependent. The altered behavior of D. melanogaster females is abolished if males lack sex peptide in the seminal fluid suggesting that SP, a male accessory gland (MAG) product, is the molecular switch. We have also studied behaviour -modifying peptides from the MAG of Aedes aegypti and showed that MAGs are an important source of head peptide (Aea-HP-1), first characterised by from mosquito heads and shown to inhibit host-seeking1. The peptide is made in the anterior portion of the gland and is transferred to the female during mating. A. aegypti often mate close to the host and therefore it is possible that male-derived Aea-HP-1 rapidly alters female host-seeking behaviour to reduce potentially lethal encounters with hosts soon after insemination. Both D. melanogaster SP and A. aegypti Aea-HP-1 are structurally designed to withstand attack by the plethora of peptidases present in insect seminal fluid. The cyclic structure of SP and the presence of blocked N- and C-termini in Aea-HP-1, confer resistance to seminal fluid peptidases. 1Matsumoto S. et al. Insect Biochem. 1989;19:277-83.

Keywords:*sex peptides, peptidases*

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The significance of PISCF allatostatins in beetles

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The insect PISCF/allatostatins (ASTs) are pleiotropic peptides that are involved in the regulation of juvenile hormone biosynthesis, are myoinhibitory on the gut and the heart, and suppress feeding in various insects, but their roles in beetles are poorly understood. To provide further insight into the significance of PISCF/ASTs in beetles, the PISCF/AST receptor from Tribolium castaneum has been characterised and its tissue distribution determined, as well as the biological activity of the T. castaneum PISCF/AST (Trica-AS). The Trica-AS receptor shows high sequence homology to other insect PISCF/AST receptors, which are related to the mammalian somatostatin/opioid receptors, a family of G-protein coupled receptors. The Trica-AS receptor was activated in a dose-dependent manner by both Trica-AS and Manduca sexta-AS, but somatostatin was inactive. Receptor and peptide transcript levels in tissues, determined by qRT-PCR, were highest in the Brain/retrocerebral complex, ventral nerve cord and/or the gut. The distribution of peptide was also shown to be widespread using an ELISA to Trica-AS, with highest levels in Malpighian tubules and , brain/ retrocerebral complex. The widespread distribution of both Trica-AS and its receptor suggest this peptide may have multiple roles in beetles. However, Trica-AS had no effect on the spontaneous contractions of the gut or ovaries of T. castaneum but this peptide did stimulate the release of enzymes from the anterior midgut of another beetle, Tenebrio molitor.

Keywords:*neuropeptide, receptor, Tribolium castaneum*

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S401M13

Development and Reproduction

Control of molt and metamorphosis in *Sesamia nonagrioides* (Lepidoptera, Noctuidae)

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Sesamia caterpillars develop in 5-6 larval instars and under short day conditions enter diapause as fully grown 6th instar larvae which then undergo up to 10 stationary larval molts. Diapause is maintained by moderate juvenile hormone (JH) titer and is readily terminated under long day conditions. In contrast to other Lepidoptera, brain is not essential driver of molt and development. On the contrary, brain removal in Sesamia caterpillars causes an increase of ecdysteroid titer. Decerebration of larvae in the 3rd or a later instar may first induce one or more larval molt(s) and/or elicit pupation followed by the imaginal molt. The incidence of induced larval molts is enhanced by the brain implants that elevate JH titer. Precocious pupation, including termination of larval diapause, occurs also in the decapitated larvae but the resulting headless pupae do not molt unless supplied with exogenous JH or ecdysteroids. Then they secrete a new cuticle combining pupal and imaginal features. Decapitated larvae develop beyond the pupal stage when implanted with the suboesophageal ganglia (SG) or with the corpora cardiaca-corpora allata complexes, whereas larvae deprived specifically of SG and retaining all other organs, molt to pupae and then their development stops. The results show that SG plays important role in the control of pupal-adult molt and that delicate hormonal interactions are crucial for proper progress of metamorphosis in Sesamia.

Keywords:*control of metamorphosis*

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S402TU01

Development and Reproduction

S402

Induction of diapause and seasonal morphs in butterflies and other insects

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The 'choice' whether to enter diapause or to develop directly has profound effects on the life histories of insects, and may thus have cascading consequences such as seasonal morphs and other less obvious forms of seasonal plasticity. Here, I briefly review recent advances regarding knowledge of the control of diapause and seasonal morphs at the genetic and physiological levels. I then use examples from my own research (primarily on butterflies) as a starting point for outlining some areas of research where we still have a limited understanding: The photoperiodic response is often modified by e.g. temperature and hostplants, but to what extent are these responses adaptive? Are growth rate differences between pathways causes or consequences? What is the role of parental effects and sex linkage on the photoperiodic control? Are seasonal morphs always adaptive?

Keywords:*photoperiod, life history, plasticity, polyphenism, seasonality, genetics*

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Possible involvement of cryptochrome and opsins in photoperiodic regulation of nymphal development in the cricket *Modicogryllus siamensis*

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The cricket Modicogryllus siamensis shows photoperiod-dependent changes in its nymphal development: Nymphs become adult within 50 days after hatching undergoing seven molts in long day conditions (LD), while in short day conditions (SD) they take much longer periods (~180 days) for nymphal development, showing an increased number of molting. Since removal of the compound eyes resulted in altered photoperiodic responses, they are a likely photoreceptor involved in the photoperiodic response. In the present study, using RNAi technology, we have examined the role of opsins expressed in the compound eye and cryptochromes in the photoperiodic response. With molecular cloning, we obtained cDNAs of three opsins, i.e. opsin-long wave (opLW), opsin-UV (opUV) and opsin-Blue (opBL), and two cryptochromes, i.e. drosophila-type cryptochrome (cry1) and mammalian-type cryptochrome (cry2). The opsin genes expressed in specific regions of the compound eye with a gene specific manner. RNAi of the opsin genes resulted in a partial disruption in the LD responses; up to 20% of the treated crickets showed a SD-like response with a prolonged nymphal period and an increased number of molting. RNAi of cry1 and cry2 also disrupted LD response in about 50% of the treated crickets. But with both opsin and cry RNAi no distinct alterations were observed in SD responses. These results suggest that the opsins and crys may play different roles in the photoperiodic response at least to LD in the cricket and they may work together for controlling proper photoperiodic responses.

Keywords:*circket, photoperiodism, photoreception, opsin, cryptochrome*

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S402TU03

Development and Reproduction

Photoperiodic response under control of circadian clock genes in the bean bug *Riptortus pedestris*

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Most organisms have evolved a circadian clock in order to anticipate daily environmental changes and many of these organisms are also capable of sophisticated measurement of daylength (photoperiodism). It has been generally accepted that the same elements are involved in both circadian (daily) and seasonal (annual) rhythms because both rely upon daily light-dark cycles. However, there remains little conclusive evidence of such molecular machinery in insects. We have approached this issue by using RNA interference (RNAi) in the bean bug Riptortus pedestris. First we verified the circadian clock genes period (per) and mammalian-type cryptochrome (cry-m) function as negative regulators, and cycle (cyc) and Clock (Clk) function as positive regulators, in the circadian clock governing the cuticle deposition rhythm of this species. RNAi directed against negative and positive regulators produced distinct cuticle layers. Simultaneously, per and cry-m RNAi caused the insect to avert diapause under a diapause-inducing photoperiod whereas cyc and Clk RNAi induced diapause under a diapause-averting photoperiod. Thus, RNAi directed against negative and positive regulators had opposite effects not only in the circadian clock but also in the photoperiodic response. This study revealed that the circadian clock operated by per, cry-m, cyc and Clock governs seasonal timing as well as the daily rhythms.

Keywords:*photoperiodism, circadian clock, RNAi*

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Transcriptomic basis of seasonal photoperiodism regulation in the pea aphid

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Aphids are one of the major pests of crops. They reproduce by parthenogenesis during spring and summer and by sexual reproduction in autumn. This switch is driven by a seasonal photoperiodism. At fall, viviparous parthenogenetic females detect photoperiod shortening and initiate the production of sexual individuals (males and oviparous females) in their offspring that will mate: fertilized sexual females will lay cold-resistant eggs that overcome harsh winter conditions. In order to describe the molecular bases involved in the regulation of seasonal photoperiodism in aphids, we developed a transcriptomic approach. We build a 7000 cDNAs microarray to study the transcriptome of heads of aphids ? where the detection and transduction of the photoperiodic signal might occur ? reared under short or long photoperiod. We could identify more than 500 different transcripts as significantly regulated in response to photoperiod shortening. Their functional classification allowed us to confirm the involvement of the neuro-endocrine system in the transduction of the signal but also to discover unexpected features such as a possible modification of cuticle structure. Indeed, the down-regulation of transcripts involved in the conjugation of dopamine and ß-alanin to form NBAD-sclerotin suggested a putative desclerotization of the cuticle under short photoperiod. Transcripts coding major enzymes involved in dopamine biosynthesis were also found be down-regulated in those conditions suggesting that dopamine levels might be decreasing under short photoperiod. A similar observation was made for transcripts involved in Insulin signaling and degradation pathways, also suggesting a possible inhibition of Insulin signalization in response to photoperiod shortening.

Keywords:*Pea aphid, Seasonal photoperiodism, Desclerotization, Dopamine, Insulin*

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S402TU05

Development and Reproduction

Arylalkylamine N-acetyltrasferase is the neuroendocrine interface regulating photoperiodism for pupal diapause of the giant silk moth, *Antheraea pernyi*

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Photoperiodism is a complex system regulated both by the circadian system and endocrine switch. Antheraea pernyi has a pupal diapause instituted when larvae developed under short-days , that can be terminated by the exposure of pupae to long-days for at least 10 cycles or incubated after a cold exposure of several months. The critical endocrine regulation for this developmental determination is whether the peptide hormone, PTTH is released or not from two pairs of neurons at the dorsolateral protocerebrum. The PTTH neurons juxtapose to the circadian neurons that express circadian proteins, Period and Doubletime. these neurons also express arylalkylamine N-acetyltransferase, NAT and NAT activity and the downstream product, melatonin show circadian fluctuation under LD cycles. NAT is activated by the exposure to long-days more than ten cycles and during chilling. We cloned nat gene and analyzed the upstream regulatory region. The upstream reagion contains several E-boxes where clock transcription modulator complex Cycle/Clock can bind.Namely nat is a ccg, clock-controlled gene. We then injected double strand RNA to knock down NAT and exposed diapause pupae to long-day condition. RNA-i worked beautifully to show photoperiodism was impaired under long day condition and no moth emerged after the injection under short-day. This proved that NAT is a critical interface of all photoperiodic clock components, circadian output pathway, photoperiodic counter and endocrine switch, as nat gene activation by circadian transcription modulator, circadian regulation of enzymatic activity of NAT, circadian regulation of melatonin and endocrine switch by melatonin binding at melatonin receptor expressed on

Keywords:*photoperiodism, N-acetyltransferase, melatonin, PTTH, circadian oscillation, Antheraea pernyi*

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S402TU06

Development and Reproduction

Endocrine mechanisms underlying loss of seasonal reproductive polyphenism in the pea aphid

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Most aphids alter the reproductive modes from parthenogenesis to sexual reproduction in response to short photoperiod (reproductive polyphenism). Although the reproductive polyphenism is thought to have acquired in early step of aphid evolution, many populations secondarily lost the sexual phase and are insensitive to photoperiodic changes. To understand molecular mechanisms of reproductive polyphenism and its secondary loss, we investigated the endocrine regulations responsible for reproductive polyphenism among several populations of the pea aphid, Acyrthosiphon pisum. First, we examined roles of juvenile hormone (JH), which was considered a likely candidate for regulating the reproductive-mode switch, and JH-related genes in aphid reproductive polyphenism. JH measurements and applications revealed that low JH titer under short day induced the sexual-morph production. Furthermore, under this condition, higher expressions of genes encoding JH-degrading enzyme were suggested to cause the low JH titer. Next, to investigate molecular underpinnings causing the evolutionary loss of sexual phase, we examined JH titer and JH-related gene expressions under long/short day in asexual populations that are though to have lost reproductive polyphenism. Neither JH titer nor JH-related genes show photoperiodic responses. Thus, it is suggested that the modification of JH degradation pathway in response to photoperiodic changes caused the loss of reproductive polyphenism in aphids.

Keywords:*phenotypic plasticity*

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Pupal color polyphenism in the swallowtail butterflies, *Papilio xuthus* and *P. machaon*

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Many butterflies show pupal color polyphenism. Environmental properties of pupation sites, such as background color, texture and curvature, affect the development of pupal coloration in many species, whereas environmental factors, such as experienced during the larval or pharate pupal stages, such as photoperiod and temperature, also affect pupal coloration in some Papilionid, Lycaenid and Pierid species. In the swallowtail butterfly, pupae of Papilio xuthus exhibit color polyphenism, such as pupae of green or brown types in non-diapause pupae, and pupae of diapause-green, orange or brownish-orange types in diapause pupae by photoperiodic condition during larval stages. Pupal coloration of this species is controlled by hormones that have been named pupal-cuticle-melanizing hormone (PCMH) and orange-pupa-inducing factor (OPIF). The former is involved the development of brown pupae in non-diapause pupae, while the latter is involved in the development of orange pupae in diapause pupae. On the other hand, P. machaon also exhibits pupal color diphenism, such as pupae of green-yellow types or brown-white types. But, there is little information about the development of pupal coloration in P. machaon. The present talk will deal with the effects of environmental factors on pupal coloration and the properties of hormonal factors affecting pupal coloration in two swallowtail butterflies P. xuthus and P. machaon.

Keywords:*Pupal color polyphenism, Swallowtail butterfly, Papilio xuthus, Papilio machaon*

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Inheritance of photoperiodic control of pupal diapause in the cotton bollworm, *Helicoverpa armigera* (Hubner)

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The cotton bollworm, Helicoverpa armigera (Hubner) enters a facultative pupal diapause in response to short-day conditions. Due to geographic variation in photoperiodic response, moths from a northern population from Langfang (39.53° N, 116.68° E) enter diapause in response to short daylengths even at the higher temperatures (D strain) whilst moths from southern population from Ledong (18° 47′ N, 108° 89′ E) exhibit almost no diapause under the same conditions (N strain). In the present study, crosses between the two strains are utilized to evaluate the inheritance of diapause under different photoperiods at temperatures 20, 22 and 25°C. The moths in both reciprocal crosses and backcrosses to D strain show a clear long-day response, similar to that of the D strain. The incidences of diapause for all F1 hybrids are intermediate between those of their parents. However, the male parent plays more important role in the determination of diapause. The N strain also shows a short-day photoperiodic response at the lower temperature of 20°C. Results from all crosses under photocycles LD 12 : 12 h or LD 13 : 11 h at 22 °C show that the capacity for diapause is transmitted genetically in the manner of incomplete dominance. Diapause duration in hybrid pupae is also influenced by their inheritance from both parents. These results reveal that both photoperiodic control of diapause induction and the duration of diapause are under the control of polygene.

Keywords:*Diapause, inheritance, Helicoverpa armigera, photoperiodic response*

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Effect of photoperiod on flight propensity and induction of adult diapause in the water strider *Limnoporus genitalis* (Hemiptera: Gerridae).

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The water strider, Limnoporus genitalis (Miyamoto), which inhabits the Sakhalin and the Kurile Islands in Russia and the Hokkaido Island in Japan, is known as a monomorphic macropter. The effect of photoperiod during nymphal stages on relative wing length (fore wing length, hind wing length / head width), wing area, wing loading, flight muscle development and take-off, and the induction of female adult diapause in L. genitalis was examined by conducting rearing trials under various photoperiodic conditions (from 10L to 17L / 24h) at 20°C. There were no differences in relative wing length or wing area of individuals reared under long-day conditions (17L-7D) compared to those reared under short-day conditions (15L-9D and 10L-14D). However, wing loading was significantly larger in females reared under long-day conditions compared to those reared under short-day conditions. The percentage of adults possessing fully developed flight muscles and taking off was significantly higher in individuals reared under short-day conditions compared to those reared under long-day conditions. Short-day conditions comprising less than 15 h light induced adult diapause in females. These results and a similar study concerning L. esakii (Miyamoto) suggested that photoperiod strongly influences the seasonality of dispersal in the water strider genus Limnoporus in Japan. Although increasing flight propensity and induction of female adult diapause under short-day conditions was common in both species, the percentage of individuals possessing fully developed flight muscles and percentage of individuals which flew more readily was higher in L. genitalis compared to the endangered species L. esakii.

Keywords:*flight muscle, wing loading, photoperiod, water strider*

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Surviving winter: Diapause syndrome in the southern green stink bug *Nezara viridula* in the laboratory, in the field, and under climate change conditions

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The southern green stink bug, Nezara viridula (L.) (Heteroptera: Pentatomidae), has long attracted attention not only as a serious pest of numerous agricultural crops but also as a species expanding its range in many parts of the world. The paper focuses on reproductive (i.e., adult) winter diapause, which is the pivotal element of the species' seasonal cycle. Results from numerous field experiments and observations, as well as laboratory eco-physiological investigations conducted during the few last decades, are analysed and interpreted. Experimental findings are used to describe the dynamics of physiological changes during overwintering. Reproductive diapause in N. viridula is controlled in both sexes by photoperiodic conditions. Induction of diapause is associated with a reversible change of body colour from green or yellow to russet (= brown). The proper timing of adult emergence and diapause induction as well as the size of adults are vitally important for successful overwintering. Nezara viridula has been shown to respond strongly to the current climate change by shifting its northern range limit, particularly in central Japan. Analysis of historical climate data suggests that, the environmental conditions during the last few decades have become more favourable for overwintering survival of N. viridula in many locations in central Japan. This has likely promoted the northward spread of the species. The relationships between reproductive diapause, reversible body colour changes, overwintering success and the recent range expansion are analysed. Perspectives of the range dynamics of the species are discussed in light of further predicted climate change.

Keywords:*Climate warming, colour polymorphism (polyphenism), dormancy, Heteroptera, photoperiodism, migration, photoperiodic response, reproduction, seasonal adaptations, winter mortality*

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A lunar rhythm derived from photoperiodic induction of diapause? Experimental dissection of lunar rhythms in the marine midge *Clunio marinus*

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In the marine midge Clunio marinus (Chironomidae, Diptera) adult emergence is timed to the tides by means of circadian and circalunar clocks. Experiments suggest that during secondary colonization of marine habitats, the circalunar clock may have derived from photoperiodic diapause: The lunar rhythm is based on a short developmental arrest of the larvae at the same stage at which photoperiodic diapause takes place. The circalunar clock is entrained via a period of nocturnal moonlight-sensitivity under the control of a circadian clock. As tidal regimes differ along the coast, C. marinus comprises many locally adapted timing races which differ in various aspects of their lunar and diurnal rhythms. Crossing experiments revealed genetic control of diurnal and lunar emergence times. Linkage mapping and quantitative trait locus (QTL) analysis identified two loci each controlling the two traits. The QTL comprise several circadian clock genes and light receptor genes. Lunar and diurnal emergence times are not inherited independently and their QTL partially overlap. This suggests that one gene may influence both traits and there may be a molecular interaction of circadian and circalunar clocks. Alternatively, evolution may have shaped genetic architecture to stabilize adaptive combinations of lunar and diurnal emergence time through genetic linkage. Currently, the C. marinus genome is being assembled and the parental strains of the crosses are screened for strain-specific polymorphisms. This may eventually reveal the first known molecular components of the circalunar clock, which may also shed new light on photoperiodic diapause induction.

Keywords:*biological clock, life cycle, lunar rhythm, QTL mapping*

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New developmental gene functions identified by a genome-wide RNAi screen in the four beetle Tribolium

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Much what we know about the genetics of insect biology is based on work in Drosophila melanogaster. However, some features of the higher Dipterans are derived, i.e. not representative for insects in general, or hard to study due to technical reasons. In addition, not all gene functions have been identified due to redundancies by lineage specific gene duplications or gene loss. A number of alternative insect model systems are being established which complement and validate Drosophila data. Tribolium castaneum stands out due to well established transgenic techniques, a strong and systemic RNAi response and a sequenced genome. In order to overcome current limitations in insect science, we perform a genomewide RNAi screen (see also http://ibeetle.uni-goettingen.de/). In this functional genomics screen we want to (1) identify missing components of well studied processes, including embryonic development. (2) we want to open new fields of insect research to functional analysis, and (3) we aim at developing Tribolium into a complementary screening platform to identify genes that will then be studied in detail in other systems. Up to date, we have injected over 4000 dsRNAs, each into both, pupae and 6th instar larvae, to identify genes with functions in embryonic development and metamorphosis. Some of the new gene functions identified in this process will be presented at the meeting. We are committed to make the functional data from this screen available to the community, and also the our library of dsRNA templates will be provided for future such screens in Tribolium.

Keywords:*iBeetle, genome-wide RNAi screen, developmental genes*

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Exploring mechanisms of embryonic patterning in *Gryllus bimaculatus*, a hemimetablous insect model system

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Mechanisms of embryonic patterning have been extensively studied in Drosophila melanogaster, a holometabolous insect. However, the mode of embryogenesis in Drosophila is highly derived and recent studies on other insects have revealed that there exist new developmental paradigms. The cricket Gryllus bimaculatus is an emerging model species for studying developmental mechanisms in hemimetabolous insects. In the past decade, RNAi-based gene function analyses greatly promoted our understanding of developmental gene networks in G. bimaculatus. Germline transformation and gene-knockout techniques have been more recently established in this species. Furthermore, we are conducting whole-genome sequencing of G. bimaculatus. Our previous studies revealed conserved and divergent aspects of the anterior-posteiror (AP) patterning system in the cricket, in comparison with other insects. Based on our recent progress, we will discuss evolution of AP patterning mechanisms in insects.

Keywords:*cricket, embryogenesis, RNAi, transgensis*

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S403TU03

Development and Reproduction

Role of Wnt and BMP signaling pathways in the regional specification of early blastoderm in the cricket *Gryllus bimaculatus*

Taro Nakamura[1], Taro Mito[2], Tetsuya Bando[3], Sumihare Noji[4]

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The mode of insect embryogenesis varies among species reflecting adaptations to different life history strategies. In Drosophila, a holometabolous insect, the Bicoid (Bcd) gradient acts as a morphogen to establish the anterior-posterior pattern. However, although Bcd is essential to anterior patterning in Drosophila, no ortholog has been isolated in any insect apart from the higher dipterans. Recent studies with these insects has been revealed that Wnt/Caudal and BMP signaling has a crucial role for AP and DV axes patterning, but how cell movement is coordinated with cell specification by inputs of these signaling pathways during the early blastoderm formation are poorly understood. To understand how the Wnt and BMP signaling pathways contribute to establishment of the DV and AP axes in Gryllus, we analyze the molecular function using parental-RNAi technique with GFP-expressing cricket. We find that the canonical Wnt signaling regulates posteriorly localization of caudal expression to specify regional identity of the posterior growth zone and that may control migration direction of embryonic cells to form a germ anlage. Furthermore, our preliminary results show that prior to AP-axis specification by Wnt signaling, BMP signaling may be involved not only in specification of DV axis but also in that of AP axis through the control of posterior aggregation of the embryonic cells during early blastoderm formation. Our data suggest that the regional specification of early blastoderm is specified by BMP signaling through the regulation of cell movement earlier than by Wnt signaling.

Keywords:*Gryllus bimaculatus, Dorsal-Ventral, Anterior-Posterior, axis specification, Embryogenesis, Short-germ insect*

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S403TU04

Development and Reproduction

The function of protein phosphatase 6 in the programmed cell death in the insect midgut

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Programmed cell death (PCD) plays an important role in insect midgut remodeling during metamorphosis. Insect midgut PCD is triggered by the steroid hormone 20-hydroxyecdysone (20E) and it is mediated by a series of genes. However, the mechanism by which 20E triggers midgut PCD is not fully understood. Here, we report a protein phosphatase 6 (PP6) from Helicoverpa armigera playing roles in midgut PCD. PP6 is expressed in the midgut during larval growth and it is significantly increased during metamorphosis. The increase is proven to be regulated by 20E. The juvenile hormone analog methoprene has no effect on PP6 expression. RNA interference analysis suggests that 20E upregulates the PP6 transcript levels through the ecdysone receptor EcRB1. PP6 knockdown by larval feeding or PP6 dsRNA injection results in the repression of the midgut PCD during the metamorphic stage. The mechanism is demonstrated to be through the suppression of genes such as Br, E74a, E75b, HR3, E93, rpr, and caspase, which are involved in 20E signaling pathway or midgut PCD. These findings suggest that PP6 is involved in the 20E signal transduction pathway and participates in the PCD in midgut. This work was supported by the grants from the National Natural Science Foundation of China (No. 30970404) and National Basic Research Program of China (973 Program, 2012CB114101).

Keywords:*Phosphatase 6, programmed cell death, 20-hydroxyecdysone, midgut.*

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S403TU05

Development and Reproduction

The evolution of dorsoventral patterning in insects

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The gene regulatory network (GRN) underpinning dorsal-ventral (DV) patterning and gastrulation of the Drosophila embryo is among the most thoroughly understood GRNs, making it an ideal system for comparative studies seeking to understand the evolution of development. With the emergence of widely applicable techniques for testing gene function, species with sequenced genomes, and multiple tractable species with diverse developmental modes, a phylogenetically broad and molecularly deep understanding of the evolution of DV axis formation in insects is feasible. I will review recent progress made in this field with particular focus on the evolution of EGF, Toll and BMP signaling.

Keywords:*axis formation, Oncopeltus, Nasonia, Tribolium, Toll signaling*

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S403TU06

Development and Reproduction

Evolution of insect thoracic morphology: Emerging views

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The first insight into the development of insect thorax and the generation of individual thoracic segmental identities were provided by now classic studies of hox genes in Drosophila some thirty years ago. These studies established the paradigm where Antennapedia (Antp) provides the default thoracic identity observed in the mesothorax (T2), whereas Sex combs reduced (Scr) and Ultrabithorax (Ubx) generate the distinct morphologies of prothorax (T1) and metathorax (T3), respectively. Because Drosophila undergoes a derived, holometabolous mode of development, we analyzed the function of these three genes in a hemimetabolous species, Oncopeltus fasciatus. The embryonic function of Antp is localized to T2 legs only, with the main effect observed in the mid-distal leg segments. However, the Antp-RNAi has no effect on the identity of the T2 segment as a whole. In a similar fashion, while Scr regulates the formation of combs on T1 legs, it does not control the overall identity of the T1 segment. This is different from the function of Ubx, which establishes the key features of the T3 segment, such as its distinct pigmentation and the repression of thoracic spiracles. These results indicate the presence of significant differences between the holo- and hemimetabolous species in regard to the development of distinct thoracic morphologies. The emerging insight from Oncopeltus also highlights the fact that the genetic mechanisms that generate the default thoracic identity (that of the T2) are yet to be determined.

Keywords:*Insect thorax, Oncopeltus fasciatus, Antp, Ubx, Scr*

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Morphological diversity of wing serial homologues in the mealworm beetle, *Tenebrio molitor*

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Of various flying animals on the earth, insects have evolved a unique flight organ. Evolutionary origin of insect wing is a long-standing question. In modern pterygota, wing appears only on meso- and metathorax. However, as insect body is composed of mutually homologous segments, the organ that is serially homologous with wing should exist in non-winged segments. Identification of such structure would provide a clue to understand origin and evolution of insect wing. In this study, we aimed to identify the wing serial homologues in the mealworm beetle, Tenebrio molitor. To identify putative wing serial homologues in non-winged segments, we examined the process of segment transformation from a non-winged segment toward a winged segment by comparing weakly and strongly affected Hox RNAi phenotypes of Tenebrio. Through such observation, we identified prothoracic hypomeron and pupal abdominal gin-traps as candidates of wing serial homologues. To obtain further support, we analyzed homologues of Drosophila key wing field regulators, vestigial (vg) and scalloped (sd). Surprisingly, in situ expression analysis and RNAi functional analysis of Tenebrio vg and sd homologues revealed that both genes play crucial roles in hypomeron and gin-trap formation, as in wing formation. These data suggests that prothoracic hypomeron and abdominal gin-traps are serially homologous with wing. From this study, insect wing serial homologues could be redefined as the source of diverged adaptive structures such as hypomeron, gin-trap and wing.

Keywords:*Wing, Serial homology, Hox, vestigial, scalloped*

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Dachsous/Fat signaling via Hippo/Salvador/Warts pathway regulates cell proliferation and pattern formation during leg regeneration in the cricket

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Regeneration depends on the recognition of tissue loss and the subsequent restoration of the relevant structure. The two-spotted cricket Gryllus bimaculatus is hemimetabolous insect and restores lost part of leg in epimorphic manner during several molts subsequent to amputation. Pattern formation of cricket regeneration legs along proximodistal axis is established by Hedgehog, Wingless, Decapentaplegic and Egfr signlaing, however, numerous questions remain, including how they detect positional disparity along the proximodistal axis, how they accurately restore the leg to its normal size, and what factors govern blastemal cell proliferation in the correct proportions. To address these questions, we have studied the molecular biological basis of leg regeneration in the Gryllus bimaculatus revealed by RNAi. We focused on genes involved in the Dachsous/Fat signaling pathway, which have been recently implicated in cell proliferation, planar cell polarity and proximodistal patterning. We found that Dachsous/Fat signaling regulates cell proliferation in blastema and in host stump and regulates positional information along proximodistal axis during leg regeneration. Since many molecules involved in cricket leg regeneration are conserved in vertebrates, our findings will provide cues to understand the longstanding questions on regeneration in metazoa.

Keywords:*regeneration, Gryllus bimaculatus*

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Freeze tolerance in drosophilid flies: A comparison of two species, the fruit fly and the malt fly.

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Insect cold tolerance represents a complex of impressive strategies. A whole array of biochemical and physiological adjustments counteract damaging effects of subzero temperatures, e.g. accumulation of low molecular weight cryoprotectants, synthesis of antifreeze proteins, remodeling of cellular membranes, and accumulation of heat shock proteins. Some insect species display the highest known levels of cold tolerance among all animals. For instance, larvae of the malt fly, Chymomyza costata (Drosophilidae) survive after submersion in liquid nitrogen (-196?C) in a fully hydrated state. We found that the principles underlying freeze tolerance in malt fly larvae are transferable, at least partially, onto a chill susceptible larvae of fruit fly, Drosophila melanogaster. Given the fact that the evolutionary adaptation for freeze tolerance is generally considered as highly complex and uneasy to mimic in laboratory, our recent results are encouraging and may open a new direction of research on long-term cryogenic storage of biological material. So far, cryogenic protocols have been developed for embryos of several insect species, including D. melanogaster. Despite indisputable progress, methods for crypreservation of insect embryos were not widely implemented into practice. The long term cryogenic storage of complex animal tissues or whole organisms still remains a great challenge. In these respect, detailed analysis of C. costata model appears as greatly promising.

Keywords:*Drosophila, Chymomyza, freeze tolerance, cryopreservation, diapause, proline*

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Insect cold storage technology: Design and practical applications

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Most insects used in research, pest control or other beneficial applications have relatively short shelf-lives. Unless a particular species has the inherent capacity to enter some type of dormancy, colony maintenance is usually by continuous culture. Continuously rearing insects in small numbers as for research can be expensive and time consuming. Rearing insects in large numbers as for pest control or pollination of beneficial crops is subject to numerous production problems. Development of insect storage technology can help alleviate many difficulties associated with nonstop insect rearing. With this motivation, our research group conducts studies with the purpose of devising short- and long term storage methodology for a variety of insects. For short-term storage, the most cold-tolerant growth stage is stored at a non-freezing temperature which promotes developmental quiescence. Recent work with solitary bees shows that a fluctuating thermal regime allows greater storage time and more quality insects than a constant temperature regime. Methods using liquid nitrogen cryopreservation have been developed for long-term storage of 6 dipteran species and one lepidopteran. Transfer of insect cryopreservation technology has resulted in preservation of 10 genetically-specific strains of screwworm embryos representing 25,000 individuals. Current projects involving formation of embryo cryobanks for insect production facilities have progressed to where protocols for several species have been upgraded to process >2000 embryos for each run with a potential of cryopreserving >10,000 embryos per 8 hr period.

Keywords:*Insect storage, cryopreservation*

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Transcriptome sequencing reveals molecular components of diapause preparation in the Asian tiger mosquito, *Aedes albopictus*

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The Asian tiger mosquito, Aedes albopictus, is currently among the most invasive mosquitoes in the world. This species is also of particular public health concern due to its ability to efficiently transmit Dengue fever, Chikungunya, West Nile and a variety of other arthropod-borne viruses. The photoperiodic diapause of Ae. albopictus is a critical ecological adaptation that has facilitated its rapid spread throughout temperate habitats. We have utilized studies of life-history variation to identify adaptive changes to the diapause response during invasion and range expansion and reproductive trade-offs associated with diapause. Additionally, next generation sequencing approaches have been used to identify the underlying molecular physiology of diapause in Ae. albopictus. Our results show rapid evolution of diapause timing during the range expansion of Ae. albopictus in North America. Next-generation sequencing approaches and quantitative RT-PCR have identified transcriptional differences in lipid metabolism genes contributing to diapause-associated reproductive tradeoffs. Next-generation sequencing has also been used to identify transcriptional elements of the diapause response related to morphogenesis, additional metabolic pathways and stress response.

Keywords:*diapause, Aedes albopictus, next-generation sequencing*

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A role for insulin signaling as a regulator of mosquito diapause

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Females of the northern house mosquito, Culex pipiens, overwinter in a reproductive diapause, characterized by an arrest in development of the ovarian follicles, fat accumulation, enhanced stress resistance, suppressed metabolic rate, and a behavioral switch from blood feeding to sugar feeding. This developmental arrest has been well documented to be linked to a shut-down in juvenile hormone (JH) production, and consistent with this view is the observation that the ovarian follicles can be prompted to resume development with an application of JH. Our recent experiments compliment the earlier link with JH by investigating the role of insulin signaling in the pathway leading to diapause. Using RNAi to block the insulin receptor in nondiapause programmed females, we generate the diapause phenotype (arrested ovarian development). Application of JH will rescue this arrest, suggesting that insulin signaling is upstream of the JH pathway. RNAi directed against the transcription factor FOXO, a gene downstream of insulin, prevents diapause-programmed females from accumulating the fat reserves normally associated with diapause. Using a FOXO antibody and CHiP sequencing we have identified a number of candidate genes that are downstream of FOXO and likely contribute to generating the diapause phenotype. Experiments with additional species suggest that the insulin/FOXO pathway may be a common element in the regulation of diapause.

Keywords:*diapause, insulin signaling, FOXO, mosquito overwintering*

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Molecular mechanisms of *Bombyx mori* embryonic diapause

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Embryonic diapause of the silkworm Bombyx mori occurs immediately after the completion of mesoderm segregation and the enlargement of the two extremities of the embryo. This diapause is induced by the action of a neuropeptide hormone, diapause hormone that consists of 24 amino acid residues with amidation at the C-terminus. Diapause hormone is biosynthesized in the subesophageal ganglion of the mother, released from it through the corpora cardiacum-corpora allatum into the hemolymph and targets the developing ovaries during the mid-pupal stage. The diapause hormone signal is received by diapause hormone receptor on oolemma, transduced to the inside of oocyte and finally connected to expression of the diapause initiating gene, pnd (pigmented and non-diapausing egg), which leads to stop the embryonic cell division at the G2 phase after completion of mesoderm segregation to enter diapause. As long as these eggs are incubated at 25oC continuously, the diapause state is maintained for about one year. To break completely the diapause, exposure to 5oC for more than 60 days is required. How is the release of diapause hormone into the hemolymph controlled? How is the diapause hormone signal connected to expression of pnd? How is the diapause broken by continuous 5oC-exposure? Our recent efforts to understand these molecular mechanisms will be discussed.

Keywords:*B. mori embryonic diapause, Diapause hormone, Receptor, Diapause initiating gene, Diapause termination*

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Stress tolerance in the Antarctic midge, *Belgica antarctica*

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In polar regions tolerance of low moisture conditions is likely as important for terrestrial organisms as cold hardiness. The midge Belgica antarctica is the southernmost free-living insect. Overwintering larvae must endure months of continuous subzero temperatures and encasement within an icy substrate without access to free water. Our recent studies examined physiological and molecular mechanisms of stress tolerance in this polyextremophilic species. Heat shock proteins (Hsps) protect organisms from injury due to temperature, anoxia, desiccation, and chemical stresses. Typically these proteins are only expressed in response to environmental stress, however summer larvae express these proteins continuously--suggesting they play a critical role in maintaining the integrity of key enzymes or other proteins in the cold. Larvae also have the capacity to rapidly cold-harden (RCH) within 1 h while frozen at -5°C, the lowest temperature known for RCH induction. Water relations are intimately linked to larval cold tolerance. Larvae are remarkably susceptible to dehydration but survive losing > 70% of their osmotically active water. Furthermore, dehydration confers increased tolerance of freezing; dehydrated larvae readily survive 3 d at -10ºC, whereas hydrated larvae cannot. Osmotic challenge caused by either dehydration or immersion in seawater elevates hemolymph osmolality and increases the synthesis of sugars and polyols. Metabolomic and proteomic studies identified other common responses to desiccation and freezing. We acknowledge of the many contributions of our students and colleagues to this research and support from the National Science Foundation (ANT-0837559 and ANT-0837613).

Keywords:*freezing tolerance, heat shock proteins, desiccation, Chironomidae*

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Drying out to survive the winter: Using RNA-seq to identify genes involved in overwintering in the Antarctic midge, *Belgica antarctica*

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The Antarctic midge, Belgica antarctica, is the world's southernmost insect and t he only insect endemic to the continent. Larvae are extremely tolerant of a number of environmental stresses, most notably desiccation; larvae can tolerate up to 70% water loss under ecologically relevant conditions. This ability to dehydrate is important for winter survival, as desiccated larvae are more cold-tolerant than their fully-hydrated counterparts. Furthermore, larvae are capable of using cryoprotective dehydration, an overwintering strategy in which larvae remain unfrozen by gradually losing water until the body fluid vapor pressure equals that of surrounding ice. In this study, we explored the molecular mechanisms of both desiccation (93% RH at 4°C for 5d) and cryoprotective dehydration (gradual chilling to -3°C at equilibrium with the vapor pressure of ice) using RNA-Seq. Using paired-end Illumina sequencing, we assembled roughly ~14,000 transcripts and measured their expression in response to desiccation and cryoprotective dehydration. Both treatments had a dramatic impact on the transcriptome, as ~25% of all transcripts were differentially expressed relative to fully-hydrated controls in each treatment. The gene expression profiles were largely similar for both treatments, including differential regulation of a number of genes related to the heat shock response, cell death pathways, detoxification, and central metabolism. However, despite these overall similarities, clustering analysis indicated that the desiccation and cryoprotective dehydration groups were transcriptionally distinct. Our results are the first large-scale transcriptomics study for a polar insect and begin to unravel the molecular mechanisms of stress tolerance in perhaps the world's most extreme insect.

Keywords:*dehydration, cold tolerance, RNA-seq, transcriptomcs, Antarctc midge*

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Global analysis of hemolymph and brain metabolites during initiation and maintenance of pupal diapause in the cotton bollworm, *Helicoverpa armigera*

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Yu-Xuan Lv, Wei-Hua Xu* State Key Laboratory of Biocontrol and School of Life Sciences, Sun Yat-Sen University,Guangzhou 510275, China. *Email:xuweihua@mail.sysu.edu.cn Abstract Environment is not always suited to organism's development. Hence, insects have evolved a strategy called diapause, for resistant the harsh conditions. The cotton bollworm, Helicoverpa armigera, is an agricultural pest, and can resist rigorous conditions through entering diapause at pupal stage. Using gas chromatography-mass spectrometry (GC-MS) based metabolomic techniques, we compared the metabolites in the brain and hemolymph between nondiapause- and diapause-type pupal. Totally, 55 metabolites in the hemolymph and 52 metabolites in the brain were identified. There are 21 and 12 metabolites altered in diapause-type pupal hemolymph and brain. Moreover, several metabolites also changed during diapause initiation. Metabolites accumulated in diapause-type pupal hemolymph including β-alanine, leucine, proline, valine and phosphoric acid, etc. In addition, methionine and tyrosine were dropped in the hemolymph during diapause. However, the major of metabolites decreased in diapause pupal brain except aspartate. Finally, a cluster of amino acids (glutamate, leucine, phenylalanine, proline and valine) changed after the diapause termination. We speculated that these altered metabolites regulated energy resource pools and enhanced stress resistance for organism. Meanwhile, they may be involved in several developmental signaling to influence diapause process.

Keywords:*diapause, metabolomics, GC-MS, Helicoverpa armigera*

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Temperature effects on the prolonged egg diapause of a katydid, Paratlanticus ussuriensis

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Diapause of Paratlanticus ussuriensis eggs can be prolonged to more than one year depending on environmental conditions. Eggs can enter an initial facultative diapause in the early embryonic stage and then enter a final obligatory diapause in the fully developed embryo stage. Initial diapause is temperature-dependent; it is initiated at 20?C and breakdown at 30?C. To determine the effects of temperature on the mechanism of initial diapause of P. ussuriensis eggs, we compared weight and DNA and RNA contents of eggs that were incubated at either 20?C or 30?C for 60 days after oviposition. Both weight and total DNA of eggs were constant when incubated at 20?C but gradually increased when incubated at 30?C. Total RNA content increased over 15 days then remained at a high level when eggs were incubated at 30?C whereas RNA content was at a constant low level when eggs were incubated at 20?C. In addition, we cloned 5 heat shock protein (hsp) genes, shsp, hsp70a, hsp70b, hsp70c and hsp90, from P. ussuriensis and determined those mRNA levels in relation to diapause. Unlike other hsp genes, hsp70c was uniquely peaked at 30?C, but not at 20?C, at the time of the initiation of initial diapause. Our results suggest that high temperature interrupts the initial diapause and induces embryonic development in the eggs. Upregulation of hsp70c may be associated with inhibitory mechanism of initial diapause entrance at high temperatures.

Keywords:*Climate change, Egg, Hsp, Katydid, Prolonged diapause, Temperature*

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S405W01

Development and Reproduction

Juvenile Hormone Synthesis Undressed

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Juvenile hormone (JH) is a key modulator of metamorphosis and ovarian maturation in mosquitoes. Adult female Aedes aegypti mosquitoes show developmental and nutritionally regulated changes of JH synthesis. To understand the molecular bases of these dynamic changes we combined transcriptional studies of the enzymes of the JH pathway with functional analysis of JH synthesis and the flux of its precursors. The lipophilic character of JH, its low concentration, fragility and the tendency to bind nonspecifically to everything had made difficult its quantification. A sensitive and robust new method was developed to quantify JH and its precursor pools in biological samples. The assay is based on the derivatization of JH with fluorescent tags, with subsequent analysis by reverse phase HPLC and a fluorescent detector. Linear responses were obtained over the range of 10-20 to 1000 fmols. FA and JH III levels were reproducibly quantified from corpora allata, hemolymph and whole body of female adult mosquito (in addition, JH III and JH III bisepoxide were detected in whole body of adult Drosophila melanogaster). Nutritional deficient mosquito females had reduced transcript levels for the genes encoding JH biosynthetic enzymes and reduced JH synthesis. Our studies suggest that JH synthesis is controlled by the rate of flux of isoprenoids, which is the outcome of a complex interplay of changes in precursor pools, enzyme levels and external regulators such as nutrients and brain factors. Enzyme levels might need to surpass a minimum threshold to achieve a net flux of precursors through the biosynthetic pathway.

Keywords:*Juvenile Hormone, mosquito, Aedes, nutrition, synthesis*

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Molecular characterization of moltinism mutants of the silkworm, *Bombyx mori*

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Silkworms typically have five larval instars, but the number of larval instar varies from three to seven in "moltinism" strains. For example, precocious metamorphosis is observed in mod (dimolting, 11-27.4), rt (recessive trimolter, 7-9.0) and M3 (Moltinism, 6-24.1) strains, while extra larval molting is observed in M5 (Moltinism, 6-24.1) strains. To uncover the molecular basis of moltinism in insects, we have performed positional cloning of these loci. Here, we report the identification and characterization of the gene responsible for the mod locus. All the mod larvae underwent precocious metamorphosis at the end of 3rd or 4th instar. Methoprene application to mod larvae during the 3rd or 4th instar was able to prevent precocious metamorphosis, and most of the treated larvae successfully developed into pupae after 5th instar, suggesting that JH reception and signaling pathway are not impaired in mod larvae. We performed fine mapping of mod and were able to narrow the responsible region within ~400 kb. Notably, we found a silkworm P450 gene CYP15C1 within this region, an ortholog of cockroach CYP15A1 that catalyzes epoxidation of metyl farnesoate to juvenile hormone in cockroach corpora allata. In mod mutant, CYP15C1 gene had a premature stop codon, and the JH titer in the hemolymph at the 3rd instar was below the detectable level. These defects could be rescued when wt allele was expressed using the GAL4/UAS transgenic system, demonstrating that CYP15C1 is responsible for mod and plays an essential role in JH biosynthesis.

Keywords:*juvenile hormone, moltinism, P450, corpora allata*

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The juvenile hormone-methoprene-tolerant complex regulates maturation of the adult optic lobe and female receptivity in *Drosophila*

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Juvenile hormone (JH) has two roles: 1) prevents the switching actions of ecdysone that are necessary for metamorphosis; 2) regulates reproductive maturation in the adult. In Drosophila melanogaster, the JH receptor is encoded by two genes: germ cells expressed (gce) and Methoprene-tolerant, a duplicate of gce. Both the gce2.5K and the Met27 null mutants form viable adults with Met27 showing delayed egg maturation and reduced fecundity, but the double mutant dies at head eversion to the pupa, similarly to wild type pupae lacking the corpora allata (CAX). Both CAX and Met27 prepupae show precocious development of the optic lobe (OL) that is rescued by JH only in the CAX prepupae. In contrast, the gce2.5K null mutant shows no precocious development of the OL. Expression of Met RNAi in selected neurons shows that JH is acting in the photoreceptors to prevent the premature differentiation caused by the pupariation peak of ecdysteroids. CAX adult females are delayed in becoming receptive to courting males; exogenous JH prevents this delay. The loss of Met causes a similar delay, but the loss of Gce has no effect on this behavior. Expression of Met RNAi in brain neurons known to be involved in female receptivity also delays its onset, suggesting that JH acts via Met to promote the maturation of these neurons. Thus, although Met and Gce are redundant in maintaining viability to adulthood and preventing premature fat body development (Li et al, 2009), each also has its own functions in modulating development of specific tissues.

Keywords:*juvenile hormone, Methoprene-tolerant, germ cell expressed, optic lobe, female receptivity*

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Met-mediated JH signaling in adult mosquitoes

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Egg maturation in mosquitoes is governed mainly by two insect hormones: the sesquiterpenoid juvenile hormone III (JH-III), and the steroid hormone 20-hydroxyecdysone (20E). Deprivation of JH in newly emerged adult female mosquitoes will halt egg maturation. Interestingly, topical application of a JH analog shortly after blood feeding inhibits the normal 20E response and also impairs egg maturation. The molecular mechanisms underlying JH action remain largely unknown. We have demonstrated that the mosquito Methoprene-tolerant protein (AaMet) and a p160/SRC coactivator of the ecdysteroid receptor complex, AaFISC, are crucial for the post-eclosion activation of some JH target genes in newly emerged adult mosquitoes. AaMet and AaFISC, two bHLH-PAS transcription factors, form a heterodimer in the presence of JH. Transcriptional activation of the JH target genes results from the recruitment of the heterodimer to JH response elements in the regulatory regions of target genes. Using structure modeling and functional analysis, our preliminary studies indicate that the PAS-B domain of AaMet is essential for high affinity binding of JH. Structurally driven mutagenesis has generated several AaMet mutants that exhibit different transactivation activities in response to JH and it's analogs, ranging from abolished activation, selective response to pyriproxyfen but not JH-III and methoprene, to constitutive activation even in the absence of JH. These preliminary results indicate that our structural- functional approach is a promising tool to uncover the structural basis for Met to act as a JH-regulated transcription factor.

Keywords:*mosquito, juvenile hormone, reproduction, receptor, gene regulation*

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MET is required for maximal action of 20-hydroxyecdysone in inducing gene expression and tissue remodeling during Bombyx metamorphosis

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Little is known about how the juvenile hormone (JH) receptor, bHLH-PAS transcription factor MET, is involved in 20-hydroxyecdysone (20E) action. Here we report that two MET proteins found in the silkworm, Bombyx mori, not only mediate JH action to induce Kr-h1 expression, but also are involved in 20E signal transduction. Met is 20E responsive and peaks during molting and pupation, when the 20E titer is high. As found with results from RNAi knockdown of EcR-USP, the 20E nuclear receptor genes, RNAi knockdown of Met at the early wandering stage disrupts the 20E-triggered transcriptional cascade, preventing tissue remodeling and causing lethality during larval-pupal transition. MET recruits general transcriptional co-activator CBP/p300 to EcR-USP for transactivation via the 20E response element. Moreover, MET binds E-box in promoter regions of the 20E-response genes and directly induces their expression. Conclusively, the 20E induction of MET is required for the maximal action of 20E in inducing gene expression and tissue remodeling during Bombyx metamorphosis. We propose that MET plays a role mediating JH-20E crosstalk.

Keywords:*MET, EcR-USP-EcRE, CBP, p300, E-box, 20-hydroxyecdysone, juvenile hormone*

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Broad complex in hemimetabolan metamorphosis: The case of cockroaches

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Broad-Complex (BR-C) is a gene of the ecdysteroid signaling pathway that encodes a family of C2H2 zinc-finger transcription factors. In Drosophila melanogaster, BR-C expresses four protein isoforms, and null mutants in which none of the four isoforms is expressed remain in a prolonged larval state and never molt to pupae. In Manduca sexta, Bombyx mori and Tribolium castaneum, BR-C is expressed in the final larval stage, and this expression is essential for pupal metamorphosis. These are holometabolous models, and the only study in hemimetabolous species reports that BR-C regulate gradual morphogenesis in Oncopeltus fasciatus. We have studied the role of BR-C in the post-embryonic development of Blattella germanica, a phylogenetically basal hemimetabolous species whose BR-C gene encodes six protein isoforms. In this species, BR-C in significantly expressed in juvenile nymphal instars, whereas the expression decreases in the last one. This expression pattern parallels that of juvenile hormone (JH) production, and JH treatment up-regulates BR-C expression in the last instar nymph. These data suggest that in B. germanica BR-C belongs to the ecdysteroid cascade but is also JH-dependent. Antepenultimate instar nymphs treated with dsRNA targeting all BR-C isoforms molt to last instar, and then to adults with reduced wings. These results indicate that in cockroaches BR-C isoforms regulate progressive development of wing buds and finally wing formation. This seems the only apparent morphogenetic role of BR-C in our basal hemimetabolous model, and is also conserved in distal holometabolous species, which suggests that this is an ancestral function of BR-C.

Keywords:*Broad-complex, metamorphosis, hemimetabolous models, juvenile hormone*

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Signaling through the JH receptor Met and the repressor of metamorphosis Kr-h1 is common to insects

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We show that the bHLH-PAS Methoprene-tolerant (Met) proteins from the beetle (Tribolium), the firebrat (Thermobia) and both Drosophila paralogs (Met and Gce) bind juvenile hormone (JH III). JH mimics pyriproxyfen and methoprene are Tribolium Met agonists. Specific mutations within the PAS-B fold of Met reduce JH binding. Met mutants incapable of hormone binding fail to interact with a partner protein Taiman. The same mutation does not affect a Met-Met complex, and makes it resistant against JH-dependent dissociation. Therefore, Met requires JH bound to its PAS-B in order to leave the homodimer and to bind Taiman. Met thus establishes a new class of intracellular hormone receptors. The capacity of Met to sense JH occurs in the ametabolous Thermobia and therefore predates metamorphosis. We further show that JH signals through a 'common-core' pathway in both holometabolous and hemimetabolous insects, the latter represented by the true bug, Pyrrhocoris. In either insect type, JH requires Met to block metamorphosis during prefinal larval stages by maintaining high expression of Kruppel-homolog 1 (Kr-h1). In final-instar Pyrrhocoris larvae, a natural drop of JH titer and consequently of Kr-h1 expression permits adult morphogenesis. Depletion of either Met or Kr-h1 in the penultimate instar triggers precocious adult development instead of the final larval instar. Conversely, exogenous JH given to final-instar Pyrrhocoris larvae re-induces Kr-h1 and causes an extra larval instar, and this effect requires Met (see Figure). The anti-metamorphic function of JH, Met and its target Kr-h1 is therefore universal to insects with diverse types of metamorphosis.

Keywords:*juvenile hormone receptor, Methoprene-tolerant, Taiman, Kruppel-homolog 1, holometaboly, hemimetaboly, Tribolium castaneum, Drosophila melanogaster, Thermobia domestica, Pyrrhocoris apterus*

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Prothoracicotropic hormone signaling in prothoracic glands of the silkworm, *Bombyx mori*

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Prothoracicotropic hormone (PTTH) is the stimulator of ecdysteroidogenesis by prothoracic gland in larval insects. We investigated signal transduction events initiated by PTTH in prothoracic glands of the silkworm, Bombyx mori. Our studies showed that PTTH inhibited the phosphorylation of adenosine 5'-monophosphate-activated protein kinase (AMPK) both in vitro and in vivo. PTTH-inhibited AMPK phosphorylation appears to be reversed by treatment with LY294002, indicating involvement of phosphatidylinositol 3-kinase (PI3K) signaling. The chemical activator of AMPK (5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside, AICAR) increased both basal and PTTH-inhibited AMPK phosphorylation. Treatment with AICAR also inhibited PTTH-stimulated ecdysteroidogenesis by prothoracic glands. The mechanism underlying inhibition of PTTH-stimulated ecdysteroidogenesis by AICAR was further investigated by determining the phosphorylation of 4E-Binding protein (4E-BP) and p70 ribosomal protein S6 kinase (S6K), two known downstream signals of the target of rapamycin (TOR). It was found that upon treatment with AICAR, a decrease in PTTH-stimulated phosphorylation of 4E-BP and S6K was detected. In addition, treatment with AICAR did not affect PTTH-stimulated extracellular signal-regulated kinase (ERK) phosphorylation, indicating that AMPK phosphorylation is not related to ERK signaling. From these results, it is assumed that the inhibition in AMPK phosphorylation, which lies in an upstream signaling of PTTH-stimulated TOR signaling, may play a role in PTTH stimulation of ecdysteroidogenesis.

Keywords:*Bombyx mori, PTTH, Ecdysteroidogenesis, Prothoracic gland cells, AMPK phosphorylation, TOR, PI3K*

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Identification and characterization of Noppera-bo, a novel Halloween-class ecdysteroidogenic enzyme

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In insects, the precise timing of molting and metamorphosis is strictly guided by ecdysteroids, which are synthesized from the dietary cholesterol via several specific catalytic steps. In the last decade, a number of ecdysteroidogenic enzymes, including the Rieske oxygenase, the short chain dehydrogenase/reductase and several cytochrome P450 monooxygenases, have been identified and characterized at the molecular level. Besides these identified enzymes, here we report a novel ecdysteroidogenic enzyme, designated Noppera-bo, which belongs to the glutathione S-transferase family. noppera-bo was identified as a gene predominantly expressed in the PG of the fruit fly Drosophila melanogaster using the microarray analysis. We have isolated several noppera-bo mutant alleles and showed that complete loss of noppera-bo function mutants show embryonic lethality and naked cuticle structure. These phenotypes are typical for the Halloween-class mutants showing embryonic ecdysteroid deficiency. Importantly, the embryonic phenotypes are rescued by a topical application of 20-hydroxyecdysone, indicating that noppera-bo mutant phenotypes are primarily caused by ecdysteroid loss. A phylogenic analysis suggests that the noppera-bo family is well conserved in Diptera and Lepidoptera. We also show that an ortholog of noppera-bo from the silkworm Bombyx mori is also predominantly expressed in the PG. In addition, the overexpression of B. mori noppera-bo rescues the phenotypes of loss-of noppera-bo function animals. These results suggest that the novel Halloween-class enzyme Noppera-bo plays an essential role in ecdysteroid biosynthesis across different insect orders. Further molecular function of Noppera-bo in ecdysteroid biosynthesis will be discussed.

Keywords:*ecdysone, Steroid hormone biosynthesis, glutathione S-transferase, Halloween gene, Drosophila melanogaster, Bombyx mori*

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Ecdysone receptors: Structure and biology

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The first biochemical evidence for an ecdysone receptor was the competition between N-ethylmaleimide and 20-hydroxyecdysone (20E) observed by Michael Ashburner in support of a central role for this protein in the Ashburner model. Verification of the model led somewhat adventitiously to the cloning of the gene encoding the EcR subunit of the ecdysone receptor which we now know to be a heterodimer of EcR and the protein USP. In ecdysone receptor structures holding 20E or the phytoecdysteroid ponasterone A, the ecdysteroid is completely enclosed within the EcR ligand binding domain with it's A and B rings in the vicinity of the β-sheet and its alkyl chain in the vicinity of helix 12.The ecdysteroid binding pocket (LBP) is accompanied by a smaller enclosed cavity termed the ancillary pocket. Three regions of the LBP will be distinguished based on degree of complimentarily with the ligand and residue conservation. We are beginning to understand the biological properties of ecdysone receptors in terms of atomic structure including the preference for different ecdysteroid hormones in particular insects. A hypothesis to explain the selectivity of the dibenzoylhydrazine insecticides for the Lepidoptera will be discussed and Ashburner's classical experiments interpreted in terms of LBP structure. The phytophagous Green Vegetable Bug, Nezara viridula, exhibits an increased preference for makisterone A over 20E. The N. viridula EcR is highly unusual in presenting a Leu substitution for an Ile in region 2 of its LBP within van der Waals distance of the additional methyl group on C24 of makisterone A.

Keywords:*Ecdysone receptor*

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Two adjacent cis-regulatory elements are required for the ecdysone response of ecdysone receptor (EcR) gene in Lepidoptera

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Nuclear receptors, EcR, E75, and HR3, are key regulators in the ecdysone-inducible gene activation cascade in insects. The transcription of these genes is induced by ecdysteroid (20-hydroxyecdysone, 20E) differently, although the detailed mechanisms underlying their responses to 20E are largely unknown. We here identify the ecdysone response elements (EcREs) for BmEcR-B1, BmE75-A, and BHR3-B isoforms from Bombyx mori using an ecdysteroid-responsive cultured cell aff3. Starting from a long upstream region of each gene that showed a 20E response, we used luciferase reporter assays to identify the respective EcREs in aff3. The EcRE of BmEcR-B1 at ? 2800, which showed ~30-fold induction by 20E, comprises two adjacent elements separated by 5bp, E1 (15 bp) and E2 (21 bp), both of which are required for the 20E response. A gel shift assay showed that E1 binds to the EcR/USP heterodimer and that E2 putatively binds with some E-box (CACGTG) binding factor such as bHLH protein. The unique E1+E2-type EcRE is also functional in other two lepidopteran cell lines, and highly conserved among promoter regions of EcR-B1 in seven lepidopteran species. By contrast, both a 20 bp EcRE (~50-fold induction) for BmE75-A and 18 bp EcRE (~1500-fold induction) for BHR3-B, comprise the E1-type EcR/USP binding element, which is also highly conserved among Lepidoptera. The combination of the E2 element or other cis-regulatory element in promoter regions may explain the different 20E responses of each class of nuclear receptor genes.

Keywords:*ecdysone, Ecdysone response element, EcR, E75, HR3, nuclear receptor, Lepidoptera, Bombyx, E-box, microarray analysis, genome information, cross talk, JH, bHLH protein,*

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Local functions of the secreted insulin-antagonist Imp-L2 in *Drosophila*

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The secreted peptide Imaginal morphogenesis protein-Late 2 (Imp-L2) acts as a growth inhibitor in Drosophila by systemically controlling the activity of the most potent growth-inducing insulin, the Drosophila insulin-like peptide 2 (Dilp-2). Imp-L2 is expressed in distinct populations of cells in various tissues such as the brain, the ring gland, the gut and the fat body, raising the question whether it also acts locally at these sites. We demonstrate that locally increasing Imp-L2 expression in the brain using a specific Imp-L2-Gal4 driver results in a developmental delay. Conversely, development is slightly accelerated in Imp-L2 mutants. These effects on developmental timing are due to alterations of ecdysone production by the prothoracic gland (PG), which are not caused by elevated Imp-L2 levels in the hemolymph but most likely result from increased Imp-L2 production in neurons targeting the PG. Our results indicate that Imp-L2 expressing neurons act as sensors of the nutritional state in Drosophila larvae and coordinate dietary information and ecdysone production to assure proper developmental timing. Furthermore, we show that Dilp-2 is secreted at specific sites in the brain-ring gland complex, where Imp-L2 expressing cells take it up. We demonstrate that the Dilp-2 uptake is dependent on Imp-L2, and that it specifically increases insulin signaling activity in Imp-L2 expressing cells. Thus, in addition to their endocrine functions, Dilp-2 and Imp-L2 act together to locally promote insulin signaling in distinct neurons of the Drosophila brain.

Keywords:*Insulin signaling, developmental timing, brain-ring gland complex, ecdysone*

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The insulin signaling pathway is required for the death of crochet forming cells during pupal metamorphosis

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Crochets are a larval specific tissue that are used to hold food plants in lepidopterans and its forming cells degenerate during the last instar stadium. The competence of the cells to form crochets in Bombyx mori declined gradually in the first two days after the last larval ecdysis, and they died 24 hr later. Both juvenile hormone and ecdysone had little effect on the change in competence, but starvation after the final larval ecdysis prevented the decline of the competence. Therefore, nutrition was responsible for its decline, and sucrose among the nutrients was the most important. Interestingly, insulin application to the larvae fed on normal diet rescued the competence, but injection of wortmannin, an inhibitor of the insulin signaling pathway, neutralized the insulin action. The expression of the insulin receptor gene in the crochet cells of normally developing larvae gradually declined, which was paralleled to the decline of the competence, but its expression increased by ~10 fold in the starved larvae. These results indicate that unlike the established role of the insulin signaling pathway, this pathway is stimulated by the lack of nutrition and is necessary for maintaining the competence of the cells to produce crochets, which is due to part of the stimulation of insulin receptor expression by starvation. Supported by the JSPS.

Keywords:*Bombyx, insulin, nutrition*

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Developmental hourglass and the chicken and egg causality dilemma

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There is a remarkable similarity in the appearance of groups of animal species during periods of their embryonic development. This classic observation has long been viewed as an emphatic realization of the principle of common descent. Despite the importance of embryonic conservation as a unifying concept, models seeking to predict and explain different patterns of conservation have remained in contention. The hourglass model that predicts excessive constraint during the mid-embryonic phylotypic period has recently received increasing support from genomics data. We will discuss several lines of evidence, from the molecular data, through developmental networks to life-history strategies, that indicate that early animal embryos are not highly conserved. Bringing this evidence together, we argue that the nature of early development often reflects adaptation to diverse ecological niches. Finally, we synthesize old and new ideas to propose a model that accounts for the evolutionary process by which embryos have come to be conserved.

Keywords:*developmental hourglass, comparative embryology, evolution of development, transcriptomics*

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The evolution of novel and diverse mechanisms of arthropod germ cell specification

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Germ cells in many Holometabolous insects, including Drosophila, are specified by maternal deposition of localised "germ plasm" that contains molecular determinants of germ cell fate. However, basally branching Hemimetabola and other arthropods are classically described as lacking germ plasm. Using molecular markers and functional genetics, we test the hypothesis that a zygotic, inductive signaling mechanism may have been used ancestrally for germ line specification within insects and arthropods. In two hemimetabolous insects and a spider, we find that transcripts and proteins of highly conserved "germ plasm genes", including piwi, vasa and nanos, are ubiquitously, rather than asymmetrically, localized during oogenesis and early embryogenesis. In all cases, molecular identification of primordial germ cells matches classical literature descriptions of the time and place of germ cell specification in late embryogenesis. Functional studies show that maternally provided vasa and piwi are not required for germ cell formation. We discuss emerging functional evidence for an exclusively zygotic requirement for these genes, and the role of conserved cell signaling mechanisms, in germ cell specification. We also explore the possible evolutionary origins of germ plasm and the role of the oskar gene in this process., oskar is both necessary and sufficient for specifying functional germ cells in Drosophila, and was previously thought to have evolved in a last common Holometabolous ancestor. Here we show that oskar evolved before the divergence of higher insects, may have played an ancestral role in nervous system development, and that its role in germ cell specification is a recent evolutionary innovation.?

Keywords:*Oncopeltus fasciatus, Gryllus bimaculatus,Achaearanea tepidariorum, RNAi, vasa, piwi, nanos*

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The coordination of growth within and between organs in *Drosophila*

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The developmental mechanisms by which growth is coordinated among developing organs are largely unknown and yet are essential to generate a correctly proportioned adult. In particular, such coordinating mechanisms must be able to accommodate perturbations in the growth of individual organs caused by environmental or developmental stress. By autonomously perturbing the growth of the wing imaginal discs within Drosophila larvae we show that growing organs are able to signal localized growth perturbation to other organs in the body and slow their growth also. Growth rate is so tightly coordinated among organs that they all show approximately the same reduction in growth rate as the developing wings, thereby maintaining their correct size relationship relative to one another throughout development. The same phenomenon is observed among developmental compartments within the wing imaginal disc when anterior compartment is growth-perturbed relative to posterior compartment and vice versa. Recent research has demonstrated that the signal produced by the growth-perturbed discs is an insulin-like peptide (dILP8). Here we present data to demonstrate that ecdysone is also a key player in coordinating organ growth. Our data suggest that ecdysone and insulin signaling work together to synchronize growth and development among organs.

Keywords:*robustness, insulin signaling, organ growth, imaginal discs, ecdysone, growth rate*

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Comparative systems-level studies of appendage morphogenesis using the crustacean model *Parhyale hawaiensis*

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A major challenge in biology is to understand how dynamic patterns of gene activity control dynamic cell behaviors to generate organ diversity during development and evolution. We have focused on arthropod appendages that develop particular shapes and sizes to suit the lifestyle of the organisms. Among arthropod models, the crustacean Parhyale hawaiensis offers a unique capacity for microscopic live imaging and tracking of all cells of developing appendages continuously from early specification up to late differentiation. Parhyale embryos undergo direct development from fertilized eggs into miniature adults within ten days, they are transparent and amenable to all sorts of embryological and functional genetic manipulations. Most importantly, each embryo exhibits a remarkable morphological gradation along the anterior-posterior body axis developing a series of different types of appendages adapted for specialized functions. This offers the great advantage to study how morphogenetic mechanisms are deployed differentially in these neighboring growing appendages to produce their differences in size, shape and pattern. Using Selective Plane Illumination Microscopy, we image appendage development at single-cell-resolution in transgenic Parhyale embryos expressing fluorescent tracers. Using these time-lapse recordings, we generate and compare the cell lineages of serially homologous appendages and quantify the cell behaviors that that account for their morphological differences. Finally, we plan to investigate at single-cell resolution with engineered BAC reporters how developmental regulatory genes guide distinct cell behaviors to control appendage morphogenesis. Our integrated approaches allow to study tissue and organ morphogenesis at many levels of biological organization, from genes to cells to final organ structure.

Keywords:*appendage morphogenesis, bioimaging*

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Rapid physiological evolution following habitat invasions

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A fundamental unresolved problem regards constraints on adaptation to novel environments. Invasive species provide valuable models to address this problem, as many are relatively unconstrained in penetrating habitat boundaries. Within the past century, the copepod Eurytemora affinis has invaded freshwater habitats multiple times independently from saline sources. To dissect evolutionary responses during these independent habitat invasions, my laboratory has been integrating analyses of physiological function with comparative functional genomics. We analyzed evolutionary shifts during invasions for pairs of ancestral saline source and derived freshwater populations across four independent invasions from two genetically distinct clades. We found evolution of increased body regulation accompanying freshwater invasions, as evinced by evolution of increased hemolymph osmolality in the freshwater populations. Physiological data revealed shifts in ion retention/integument permeability and changes ion-motive ATPase activity in freshwater relative to saline populations. Most notably, ancestral saline populations selected for freshwater tolerance in the lab recapitulated the adaptive shifts in V-type H+ ATPase activity observed in natural populations. V-type H+ ATPase localization and activity has been hypothesized to be critical for the invasion of fresh water, and perhaps land, but no previous study had demonstrated evolution of function of V-type H+ ATPase. Moreover, the repeatability of evolutionary events, across multiple independent invasions in the wild and selection experiments in the laboratory, indicates the evolutionary lability of this trait. Our findings are consistent with the evolution of increased physiological regulation accompanying transitions into stressful environments.

Keywords:*osmoregulation, adaptation, salinity, biological invasions, biogeography*

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Development and Reproduction

The development and evolution of the exaggerated weapons of sexual selection

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The morphological diversity of insects is one of the most striking phenomena in biology. Evolutionary modifications to the relative sizes of body parts are responsible for a vast range of body forms. Traits with exaggerated proportions such as the "weapons" of beetles have evolved multiple times among several scarab lineages. Sexual selection has shaped the evolution of male phenotypes in many species in which only the males develop weapons that they use to combat rival males for access to females. As with other sexually-selected traits, many beetle weapons are phenotypically plastic resulting from differences in the nutritional environment experienced by growing larvae. What remains unknown however, are the mechanisms that are responsible for coupling nutrition with the disproportionate growth of beetle weapons. Here, we demonstrate the significant roles of endocrine pathways for coupling nutrition with growth, from two case studies in horned beetles ? the Asian rhinoceros beetle Trypoxylus dichotomus and the stag beetle Cyclommatus metallifer. In T. dichotomus, we focus on the insulin signaling pathway as a candidate pathway for condition-dependent growth. RNAi gene-knockdown of the T. dichotomus insulin receptor (InR) resulted in a significant decrease in the weapon size of males. In C. metallifer, another important insect endocrine factor, juvenile hormone (JH) was analyzed. JH titer measurements revealed positive correlations between prepupal JH titer and future mandible size of males. Ectopic application of JH analog to the male stag beetle induced spectacular mandible- specific growth resulting in disproportionate overgrowth of this structure compared to other morphological traits.

Keywords:*Exaggerated morphology, Juvenile hormone, Insulin signaling, weaponed beetle, phenotypic plasticity*

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Population dynamics of populus leaf miner *Lithocolletis populifoliella* Tr. in

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Lithocolletis populifoliella Tr. is one of the most important pests of Populus plantations in West Azerbaijan province. Activity of this pest has been noticeable on different clones of Populus species, during recent years. This survey was carried out in order to determine the density and activity of L. populifoliella on 10 clones of Populus (Populus nigra and P. eauramericana) in Populus research station of Mahabad, during 2009 and 2010. The clones were cultured in RCBD (within 3*3 meters distance), in three replications (75 samplings from each clones in three replications). From early April to late September every 10 days and, due to removal of additional effects, 16 samplings out of 25 in each replicate were selected. The number of L. populifoliella larvae was counted on four thirty-centimeter-long branches in four cardinal directions. Statistical analysis indicated significant difference between treatments in terms of number of larvae on the Populus clones. Results showed that the highest and the lowest density of L. populifoliella were recorded on Populus nigra 63/135 and P. eauramericana 488, respectively. This pest produces two generations per year in West Azerbaijan province. The peak of population in 2009 was observed in mid-June for the first generation and mid-July for the second generation of this pest. The peak of population in 2010 due to warm condition for the first and second generations of L. populifoliella was observed in early and late June, respectively.

Keywords:*Populus spp., Pest, Density, Population*

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Diverse developmental and genetic mechanisms direct convergent evolution of a reproductive fitness trait in *Drosophila*

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Convergent morphologies often arise due to similar selective pressures in independent lineages. It is poorly understood whether the same or different developmental genetic mechanisms underlie such convergence. Here we show that independent evolution of a reproductive trait, ovariole number, has resulted from changes in distinct developmental mechanisms each with a different underlying genetic basis in Drosophila. Ovariole number in Drosophila is species-specific, highly variable, and largely under genetic control. Convergent changes in Drosophila ovariole number have evolved independently multiple times within and between species. We show that the number of a specific ovarian cell type, terminal filament (TF) cells, determines ovariole number. We further examine TF cell development in different Drosophila lineages that independently evolved a significantly lower ovariole number than the D. melanogaster Oregon R strain. We show that in some lineages, fewer somatic gonad precursors are established during embryogenesis, resulting in lower ovariole number. In contrast, in other lineages, changes in somatic gonad cell morphogenesis during larval development lead to changes in ovariole number. Evolution at the InR and bab loci has been implicated in previous analyses of genetic changes associated with evolutionary variation in ovariole number. We show that mutations in the D. melanogaster Insulin Receptor (InR) alter gonad primordium size but not ovarian morphogenesis, while targeted loss of function of bric-a-brac 2 (bab2) affects morphogenesis without changing gonad primordium size. Thus, evolution can produce similar ovariole numbers through distinct developmental mechanisms, likely controlled by different genetic mechanisms.

Keywords:*convergent evolution, morphogenesis, fecundity, ovariole, terminal filament*

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Cytoneme-mediated delivery of hedgehog regulates the expression of bone morphogenetic proteins to maintain germline stem cells in *Drosophila*

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Stem cells reside in microenvironments, or niches, which often contain support cells that control stem cell maintenance and proliferation. Hedgehog (Hh) proteins mediate homeostasis in several adult niches, but a detailed understanding of Hh signalling in stem cell regulation is lacking. Studying the Drosophila female germline stem cell (GSC) niche, we show that Hh acts as a critical juxtacrine signal to maintain the normal GSC population of the ovary. Hh is produced in cap cells, a type of niche support cells and it is then secreted to a second, adjacent population of niche cells, the escort cells, where it activates transcription of the GSC essential factors Dpp and Gbb. In wild-type niches, Hh protein decorates short filopodia that originate in the cap cells and that are functionally relevant, as they are required to transduce the Hh pathway in the escort cells and to maintain a normal population of GSCs. These filopodia, reminiscent of wing disc cytonemes, grow several fold in length if Hh signalling is impaired within the niche. Because these long cytonemes project directionally towards the signalling-deficient region, cap cells sense and react to the strength of Hh pathway transduction in the niche. Thus, the GSC niche responds to insufficient Hh signalling by increasing the range of Hh spreading. Although the signal(s) perceived by the cap cells and the receptor(s) involved are still unknown, our results emphasise the integration of signals necessary to maintain a functional niche and the plasticity of cellular niches to respond to challenging physiological conditions.

Keywords:*Drosophila, niche, germlline stem cells, hedgehog, dpp, gbb, cytonemes*

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Laminins are required for proper formation and maturation of eggs in *Drosophila*.

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Drosophila eggs arise from multicellular units known as egg chambers. Each egg chamber consists of a germline cyst surrounded by an epithelium of somatic follicle cells (FC), the follicular epithelium (FE). Egg chambers are initially spherical and elongate dramatically along their anterior-posterior (A-P) axis as they develop. This shape change is important to allow mature eggs to be laid. The elongation process coincides with a planar polarization of the FE and the formation of parallel arrays of actin bundles at the basal surface of FCs. These bundles align with one another perpendicular to the A-P axis, mirroring components of an extracellular matrix (ECM) in the adjacent basement membrane. Egg chambers spin around their A-P axis during elongation, which is required to lay down the polarised ECM. The current model is that this circumferential organization of structural molecules around the egg chamber acts as a molecular corset to promote elongation. Mutations in ECM components, such as Collagen IV or Perlecan, and their receptors, such as integrins, result in round eggs, demonstrating that cell-ECM interactions are required for egg chamber elongation. However, the molecular and cellular mechanisms by which these interactions control egg chamber elongation remains poorly understood. Here, we provide evidence for a function of laminins in the formation and maintenance of a basal polarised ECM and in the proper spinning of egg chambers. These findings highlight a role for the ECM in shaping tissues, which is critical for many morphogenetic processes, such as the formation of mature eggs.

Keywords:*extracellular matrix, egg morphology, sterility*

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Development and Reproduction

Steroids and miRNAs cooperate in regulation of the stem cell niche

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The adult stem cells in the female ovary of Drosophila provide an excellent model to study germline stem cell niche interactions. Previously it has been shown that in Drosophila steroid hormones are required for the progression of oogenesis during later stages of egg maturation. Furthermore ecdysteroids regulate niche formation and the early differentiation of a germline stem cell daughter. In the absence of these hormones, the developing cystoblasts are blocked in a single cell state which can be overcome by ecdysone supply. Interestingly, ecdysone signaling cell-autonomously modulates the strength of TGF-β signaling in the germline, and also non-autonomously coordinates crosstalk between germline and escort stem cell progeny via cell adhesion. The specificity of this systemic signaling is achieved as a result of spatial expression of EcR/USP co-activators and repressors. Now we also show that the response to ecdysone signaling is modulated via miRNAs. Not only is the expression of these miRNAs dependent on ecdysone signaling during development, but in turn miRNAs target tissue specific ecdysone co-regulators in a regulatory feedback loop.

Keywords:*miRNAs, ecdysone, stem cell niche, germline stem cell*

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Hormonal coordination of niche formation with germ line stem cell establishment

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In a Drosophila ovary, somatic niches and germ line stem cells (GSCs) form functional units that continually produce eggs. We show that ecdysone receptors coordinate GSC unit formation. In particular, ecdysone is required for niche formation, and at the third larval instar for the differentiation of primordial germ cells (PGCs, the precursors of GSCs) into germ line cysts. Thus, similar to the mammalian hypothalamus-pitutiary-gonadal axis, the initiation of oogenesis in drosophila depends on a brain-gland-gonad axis. The ecdysone receptors EcR and Usp are expressed in the somatic cells of larval ovaries. At early larval stages, EcR and Usp repress somatic niche formation and PGC differentiation. This early repression allows both niche and GSC precursors time to proliferate, and is required for correct positioning of larval GSC units. The early repressive function of EcR and Usp results in repression of the Broad-Z1 isoform. At late larval stages, EcR and Usp are positively required for Broad-Z1 expression, for niche formation, and for PGC differentiation. In broad mutants, or upon somatic expression of a dominant negative form of EcR, niches and PGCs fail to differentiate. Temporal studies reveal that ecdysone affects PGCs and niches sequentially, and in parallel. In summary, ecdysone receptors are major coordinators of ovary formation by their dual role as early repressors, and later as activators of Broad expression. ecdysone signaling in the ovary is one example of how a single signaling pathway might control niche formation, establishment of its resident stem cells and the initiation of their differentiation.

Keywords:*ecdysone, germ line stem cell, niche, oogenesis*

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S407F06

Development and Reproduction

Insulin signaling: A tangled web controlling reproduction and aging in the mosquito

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The insulin signaling cascade regulates a variety of physiological processes in insects, including metabolism, lifespan, immunity and reproduction. In the mosquito, an understanding of how insulin signaling regulates these processes could provide us with novel target for controlling disease transmission. The role of insulin signaling on mosquito reproduction occurs at multiple levels. Insulin signaling has been implicated in controlling steroidogenesis in ovarian follicle cells and vitellogenesis in the fat body. However, the reproductive role of insulin signaling in the midgut, if any, has not been elucidated. To further assess the role of insulin signaling on mosquito reproduction we genetically engineered Aedes and Anopheles mosquitoes with increased insulin signaling in the fat body, midgut and ovary. This was accomplished by inserting a myrstoylated form of Akt regulated by the vitellogenin, carboxypeptidase, or an ovary specific promoter respectively. Reproduction was assessed during multiple blood meals between transgenic mosquitoes and non-transgenic sibling controls. Mosquitoes overexpressing myrAkt in the midgut or fat body had no significant change in egg production, although vitellogenin transcript expression increased in the fat body. However, mosquitoes overexpressing Akt in the ovary had a small, but significant increase in egg production. By modifying insulin signaling in key reproductive tissues it may be possible to manipulate the mosquito's life history, including reproduction and lifespan.

Keywords:*Akt, lifespan, Aedes aegypti, Anopheles stephensi*

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The Hippo pathway in panoistic ovaries

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Drosophila melanogaster, the classical model of meroistic ovaries, has been deeply studied in the past years. Hovewer, few information is available about the molecular mechanisms regulating oogenesis in panoistic ovaries. In an attempt to afford data on this primitive ovarian type, we work on Blattella germanica, a cockroach species having panoistic ovaries where only the basal oocyte is developed in each gonadotrophic cycle. In the long term, our interest is to understand the evolutionary transition between panoistic and meroistic ovary types. As the hippo pathway is conserved in all organisms studied to date, it can be a suitable model to afford data on the evolution of these two ovary types. Ovarian transcriptomes obtained in our research group throws us most of the components of the hippo pathway, and we have studied the mRNA expression pattern, and the function of the hippo core pathway in the regulation of the follicular epithelium using RNAi techniques. Our results show that in ovaries with hippo components depleted, the stalk cells in the follicle lost their fate, probably by affecting Notch signalling. In addition, in the follicular epithelium we observed cell over-proliferation and the formation of a double layer of cells. These results are consistent with the previous studies in the meroistic ovary of D. melanogaster, thus suggesting that the function of hippo pathway is generally conserved in insect ovaries.

Keywords:*Blattella germanica, polarity, cell proliferation*

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Development and Reproduction

Designing of a virus-regulated mosquito lethal gene

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Dengue fever ranks as the most important mosquito-borne viral disease in the world. Incidence has increased 30-fold in the last 50 years. An estimated 2.5 billion people live in endemic locales and as many as 50 million infections occur annually with 500, 000 cases of dengue hemorrhagic fever and 22,000 deaths. The number of countries reporting dengue hemorrhagic fever has increased more than four-fold since 1970. Vaccines to protect human populations from dengue infection do not exist and insecticide resistance has hampered efforts in vector control 1. These circumstances emphasize the urgent need for alternative approaches to control the transmission of dengue worldwide. The goal of this project was to generate transgenic Aedes aegypti mosquitoes that die upon infection with dengue virus, interrupting virus replication and transmission. This species is the major vector in the majority of dengue epidemics2. These mosquitoes will be of value to public health programs charged with controlling dengue epidemics. The key components of this strategy are the NS3 viral protease, a heat=shock minimal mosquito promoter, and an engineered anti-dengue gene (ADG). The ADG encodes a fusion protein with a transmembrane-endoplasmic reticulum (ER) localization signal, a cleavage site recognized by the viral protease and the complete open reading frame (ORF) of michelob_x (mx), the mosquito orthologue of the reaper/grim-like inhibitor of apoptosis protein (IAP) antagonist genes that have a pivotal role in cell death regulation in Drosophila. The 3'UTR of the transgene will include a SV40 polyadenylation signal to stabilize the transcript.

Keywords:*transgenic mosquitoes, dengue, Aedes aegypti*

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Promotor sequences of two vitellogenin genes and transcription regulations by JH/20E and endocrine disruptors

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The American cockroach, Periplaneta americana has two vitellogenin genes, Vg1 and Vg2. We analyzed the promotor sequence of these genes and identified transcription mediator binding sites by sequence analyses and Luciferase reporter construct: the 935 bp Vg1 promotor contained 1 RXR, 1E74A, 3 JHRE, 2 E75, 1 EcRE and DR2 sites, while the 1804 bp Vg2 promotor contained 2 E74A, 4 JHRE, 1 RXR, 1E75, 2 EcREand 2 DR2.The reporter constructs showed the effect of JHIII and 20 E on the transcrpition activities for both vitellogenin genes. JHIII was stimulatory but 20E was in general inhibitory.Since there are other lipid factor- and transcription momulator-binding sites are present, there is a possibility that some endocrine disruptors may bind these enhancer silencer elements. The Vg1 promotor has an-E box that is a binding site of PAS proteins that include Methoprene resistant(Met) through whicj JHIII may acts. PAS stands for Per-Arnt-Single-minded and Arnt is a arylhydrocarbon receptor (AHR)-nuclear translocator. Therefore, this class of endocrine disruptors may bind the E-box, as well as the circadian transcription regulators such as CYC or CLOCK may also affect.We have already shown that four representative endocrine disruptors siverely affect the vitellogenin gene transcription in the giant water bug, a red-data species in Japan (Nagaba et al., 2011 J.Insect Conservation 15, 421) and now examine the possibility of using this promotor for monitoring environmental pollutants that work as endocrine disruptors as well as JHIII receptor.

Keywords:*Vitellogenin gene 1(Vg1), Vg2, JHIII, 20E, endocrine disruptors, environmental sensor*

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Development and Reproduction

Cell competition and compensatory cellular hypertrophy in nonproliferating tissue

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Cell competition, a struggle for existence between cells in heterogeneous tissues, is an intrinsic homeostasis system through which normal cells sense and remove aberrant (e.g. precancerous) ones, to maintain tissue integrity in multicellular organisms. Elimination of suboptimal loser cells is accompanied by 'compensatory proliferation' of winner cells. As a result, cell competition not only eliminates potentially deleterious cells during development but also assures that final organ size is properly controlled. So far, cell competition has only been found in tissues where cells undergo proliferation. Speed of proliferation is generally accepted as the primary criterion determining winner and loser cells, but here we report that cell competition can also occur between nondividing cells with different genetic backgrounds. In nonproliferating Drosophila follicular epithelia, we found that Minute heterozygous or mahjong homozygous mutant cells were eliminated by their wild-type neighbors through cell competition. The winner cells did not undergo compensatory proliferation but instead underwent 'compensatory cellular hypertrophy' to compensate loss of loser cells. This hypertrophy was implemented by acceleration of the endoreplication cycle. Moreover, we found that elimination of the loser cells required upregulation of JNK signaling and that endocycle acceleration involved upregulation of insulin/insulin-like growth factor-like signaling, both in the winner cells. These studies revealed a novel tissue-homeostasis strategy in nondividing tissue: elimination of potentially deleterious cells through cell competition and repair of the tissue through compensatory cellular growth

Keywords:*cellular hypertrophy*

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Micro RNAs in the control of the differential development of *Apis mellifera*

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Apis mellifera queens and workers are prime examples of how deeply the environment could affect ontogenesis. In bees, a differential protein-containing diet is responsible for the high levels of juvenile hormone (JH) observed in queens, which, in turn, directs larval development and the morphological differences observed in both castes. The differential levels of JH is controlled by a specific enzyme the juvenile hormone esterase (JHE). The JH activation of physiometabolic gene cascades allowed queens to grow differentially and be an efficient egg laying machine. However, in the presence of low levels of JH, honeybee workers develop characters transforming them in highly specialized member of the colony, active pollinators with extraordinary cognitive capacity. Aiming to shed some light on JHE role in caste development and differentiation we interfered its codifying gene using RNAi. We conducted an experiment treating 3 groups of young larvae (L2) with Amjhe dsRNA and leaving other 3 groups developing without treatment, both until pre-pupal phase. The miRNA of the six groups were deep sequenced using Illumina Technology. We observed that the miRNA gene family differ profoundly between the treated and untreated groups. We selected 14 miRNAs and searching for their targets (RNAHybrid) we found approximately 1100 candidates (affected genes). Looking for the Ortologs we note that preferentially developmental genes were affected, indicating that the more expressed miRNA are inhibiting the characteristic worker developmental genes directing the development to queen like. Confirming our hypothesis that JHE is in the regulation of caste differentiation. Financial support: FAPESP

Keywords:*Apis mellifera miRNA*

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The influence of juvenile hormone on the social physiology of bumble bees (*Bombus terrestris*)

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Juvenile hormone (JH) signaling is inhibited in the presence of the queen in both honey bees and bumble bees. However, in honey bees, JH regulates age- related division of labor whereas in bumble bees JH titers are correlated with ovarian development. Thus, the pathways downstream JH are predicted to at least partially diverge in order to accommodate the different functions in the two species of bees. We found that allataectomized Bombus terrestris workers do not develop their ovaries even under queenless conditions that typically promote worker reproduction. This effect was reversed by replacement therapy, confirming the gonadotropic function of JH. Similar manipulations of JH signaling also influenced metabolism, wax secretion, and fat body development. Hemolymph JH titers did not differ between nurses and foragers suggesting that JH does not regulate division of labor in bumble bees. We further examined the expression patterns of a transcription factor, Kruppel-homolog 1 (Kr-h1) in bumble bees. Kr-h1 expression is downregulated in honey bee workers upon exposure to the queen pheromone, and upregulated in foragers. In the bumble bee, Kr-h1 levels were similarly downregulated in the presence of the queen or dominant workers. Kr-h1 levels were also associated with JH-mediated changes in reproductive state, but elevated levels were not associated with foraging behavior. These results suggest that Kr-h1 is a part of a JH signaling pathway that is important for social regulation, but was differentially co-opted during the social evolution of bumble bees and honey bees.

Keywords:*juvenile hormone, bumble bee, honey bee, reproduction, Kruppel-homolog 1, oogenesis, foraging*

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Transcriptional control of ovary size - cell death in the ovary of honey bee worker larvae

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Divergent development of the ovary phenotype of honey bee queens and workers starts from a bipotential larval state, with each ovary comprising 150-180 ovariole primordia. In queens, all develop into functional ovarioles, whereas programmed cell death in the fifth larval instar eliminates 95-99% of these in workers. To understand underlying molecular mechanisms we generated suppression subtractive hybridization libraries, giving a total of 136 unique sequences. 72 % of the queen library sequences represented known or predicted genes, leaving 28% as unpredicted, in contrast with the worker library where 59% represented unpredicted genes. A set of 16 sequences was chosen for expression analysis by quantitative RT-PCR. Statistically significant expression differences were denoted for a short chain dehydrogenase/reductase gene overexpressed in workers, an ornithine amino transferase gene overexpressed in queens, and two ESTs representing unpredicted genes, both located in chromosome 11. Complete CDSs for the latter, obtained by 3'5'RACE, revealed that neither contained an ORF of significant length. RNA secondary structure analysis confirmed both as putative long non coding RNAs, these named lncov1 (overexpressed in workers) and lncov2 (overexpressed in queens). In the honey bee genome, lncov1 maps within an intron of a predicted gene of unknown function, whereas lncov2 is intronic to fringe. Importantly, both genes are located within the previously identified chromosome 11 QTL for ovariole number variation in bees. This is the first evidence for a potential role of long non-coding RNAs in the developmental regulation of a socially relevant reproductive trait. Financial support by FAPESP

Keywords:*Apis mellifera, social insect, ovary, differential gene expression, long non-coding RNA*

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The i5K pilot genome sequencing at the Human Genome Sequencing Center.

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The i5K is an initiative to sequence the genomes of 5,000 arthropods of medical, agricultural and scientific importance. As a pilot project to identify potential problems and challenges in species selection, identification and acquisition, DNA isolation, sequencing strategies and assembly, automated and manual annotation, analysis and publication, the BCM-HGSC is sequencing ~30 arthropods, selected by the i5K species selection committee. Our extensive experience in arthropod genomics, has allowed us to quickly identify new challenges in scaling the number of de-novo genome projects. For example, many arthropod species have much larger genome sizes than initially expected. For a number of arthropod orders, small body sizes yield tiny amounts of DNA. Other challenges include the inability to inbreed, a common situation for most arthropods or sex individuals ? for example arthropods where only larvae can be easily collected. An additional challenge is to identify ongoing genome projects to avoid replication of work. This challenge will become increasingly important as the reduction in sequencing cost leads to a rapid increase in genome sequencing projects. We present our success in working with community groups to select appropriate species for sequencing, our Illumina sequencing plans, and initial assembly demonstration results, our proposed modifications for extremely low quantities of DNA, and our automated analysis plans. Also we present an outline of the i5K community annotation and analysis process. Most importantly the i5K project is establishing a genomic molecular baseline to the study of animals that affect our society so greatly.

Keywords:*i5K genomes*

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S501M02

Genetics, Genomics and Evolutionary Entomology

The arthopod genomics revolution

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The assembly of a new genome opens the door for scientific exploration on the focal species at a whole new level. Although a series of arthropod genomes have been published or close to finishing, the available genomes are significantly biased towards just a few lineages. For example, all published insect genomes are from just 6 orders, most of which belong to holometabolans. The under representation for the majority of arthropod lineages is mainly due to difficulties in current sequencing technologies and informatics in dealing with large and complex genomes. In this presentation, I will introduce the current progress in arthropod genomics research at the BGI. The genomes of a number of insect species, including those with relatively high levels of heterozygosity and repeat sequences, have been successfully assembled using a strategy combining whole genome shot-gun and fosmid-to-fosmid sequencing. In the mean time, large-scale international collaborations synergizing research expertise and resources are coordinated by the BGI. For example, a new initiative aiming to catalogue genomic features of the species and populations of global Daphnia is being developed by the BGI and the Daphnia Genomics Consortium (DGC). Biological materials, including living animal stocks, and a whole spectrum of genomic data, accompanied by detailed records of morphological/biological/ecological/toxicological traits, will be generated through this international collaboration and made available to the community. Through this effort, we hope to gain a better understanding of the genomic mechanism underling the rapid adaptation to environmental changes and evolutionary patterns in Daphnia.

Keywords:*insect, genome, evolution, function, database, BGI*

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Molecular regulatory mechanism of phase change in locusts

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The migratory locust is a notorious agricultural pest species around the world. The individuals of the locusts exhibit remarkable phenotypic plasticity, termed "phase change", in response to local population density. The gregarious locust populations often intrigue plagues and cause large economic losses in agriculture. Molecular mechanism of phenotypic plasticity that contributes to mass swarming remains unknown. Here, a genome-wide screening by oligo-nucleotide microarray was used to study the gene expressions in time-courses of solitarization and gregarization, as well as at different nymph stages in two extreme phases. Several CSP genes and one takeout gene, which have higher expression levels in gregarious and solitarious locusts respectively, significantly displayed opposite expression trends during time courses. RNA interference and olfactory behavioral experiments confirmed the CSP and takeout genes involved in the shift of attraction/repulsion behavior. Stadium-by-stadium comparison between two phases revealed that genes pale, tan, ebony and henna are more active in gregarious phase than in solitary phase, and vice versa for hexamerin and JHBP. Repression of gene sets (pale, ebony and VAT1) in dopamine metabolic process by pharmacological agents or RNA interference in gregarious hoppers induced solitary behavior, whereas L-DOPA-injected solitary hoppers exhibited gregarious-like behavior. These findings suggest that olfaction-related genes initiate the behavioral phase change and genes in dopamine pathway maintain behavioral and body color traits in the two phases.

Keywords:*phenotypic plasticity, phase change, migratory locust, microarray, olfaction, dopamine*

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S501M04

Genetics, Genomics and Evolutionary Entomology

What Nasonia Genomes Reveal about Adaptation & Speciation

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The genetic bases of morphological, developmental and behavioral differences between species are still largely unknown. Genomics now provides a means for uncovering genetic and molecular mechanisms of species divergence in diverse "emerging model" systems. A challenge still remains - going from individual quantitative trait loci (QTL) to identifying the precise genes and sequences causing a trait difference. Described here are successful efforts to identify QTL causing growth differences among species of the insect Nasonia. Nasonia is a complex of closely related species that differ in male wing size. Nasonia has genome sequences for the species, interfertile hybrids, and short generation time assist. Exploiting these genetic advantages, we have cloned two major QTL causing species growth differences. One is a QTL causing ~40% increase in wing growth and maps to the cis-regulatory region of the master sex determination gene doublesex (dsx). The second major QTL causes an increase in male wing width through increases in cell number and size. Fine-scale mapping and in situ hybridization reveals changes in at least three (non-coding) regions around the growth gene unpaired (upd) cause spatial/temporal change in expression of upd and growth of the developing wing. Upd is believed related to the growth regulator interleukin-6 in vertebrates. These findings support the view that cis-regulatory changes in functionally conserved developmental genes are targets for morphological evolution. The results also illustrate how genomic tools can rapidly advance new systems for studies of basic biological processes.

Keywords:*evolution species genetics*

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S501M05

Genetics, Genomics and Evolutionary Entomology

An integrative approach applied to the phenotypic plasticity of the pea aphid

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Sequencing a genome is a first step of a great adventure: such like anatomy which is required for understanding physiology, sequencing a genome is necessary for discovering the complex processes of genome regulation for individual and species adaptation. All these processes (anatomy and functioning of a genome) are collective and require a strong community working together. A summary and updates on the pea aphid genome story driven by the International Aphid Genomics Consortium will be given. Then, examples of what has been done in our lab using these genomic resources will be presented: we aim at integrating population genomics, quantitative genetics and transcriptomics to get comprehensive insights into the variations of reproductive mode in aphids, by linking phenotypic plasticity (molecular bases of clonal and sexual phases within a given genotype) and polymorphism (co-existence of sexual and asexual lines within a given species). We detected genomic regions that have evolved under divergent selection whether populations reproduced asexualy or sexualy one time per year. We identified 5 quantitative trait loci (QTLs) linked to the reproductive mode in the pea aphid, among which one region was also detected to have evolved under divergent selection between sexual and asexual populations. This region contains sequences related to the juvenile hormone pathway and transcriptomic analyses by RNA-Seq are underway to assess their potential involvement in the determination of reproductive strategies. These integrative approaches could not be performed without a prior join effort of a whole community.

Keywords:*reproductive mode, functional genomics, polyphenism*

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S501M06

Genetics, Genomics and Evolutionary Entomology

DNA methylation variation among morphs of the pea aphid, *Acyrthosiphon pisum*

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Aphids display extraordinary polyphenism, in which discrete alternative phenotypes are produced in response to environmental cues. From the same genetic background, various morphs are generated differing in their morphology, physiology and behaviour. In this study, DNA from aphid heads was used to compare the methylomes of three aphid morphs of the same clonal lineage (LSR1): Unwinged asexual females (viviparous apterae), winged asexual females (viviparous alatae), and unwinged sexual females (oviparous apterae). Averaged across all genes, all morphs showed similar levels and patterns of CG methylation across gene bodies, with consistently higher levels of methylation in exons and lower levels in introns. In contrast, the levels of CHG and CHH methylation were more variable among the morphs, and a pattern of increased intron-specific methylation was observed in the sexual morph. RNASeq analysis was used to associate observed differences in DNA methylation between morphs with variation in expression and splicing of individuals genes. The implications of these results in terms of the role of DNA methylation in regulating aphid developmental polyphenism are discussed.

Keywords:*polyphenism, epigenomics, development,*

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S501M07

Genetics, Genomics and Evolutionary Entomology

The genome of two spotted spider mite *Tetranychus urticae*

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The two spotted spider mite Tetranychus urticae is a cosmopolitan agricultural pest with an extensive host plant range and an extreme record of pesticide resistance. T. urticae represents one of the most polyphagous arthropod herbivores, feeding on more than 1100 plant species belonging to more than 140 different plant families including species known to produce toxic compounds. It is a major pest in greenhouse production and field crops, destroying annual and perennial crops such as tomatoes, peppers, cucumbers, strawberries, maize, soy, apples, grapes, and citrus. Our international consortium sequenced and annotated spider mite genome, representing the first complete chelicerate genome. At 90 Mb T. urticae has the smallest sequenced arthropod genome. The spider mite genome reveals unique changes in hormonal milieu, organization of the Hox complex, and evolution of silk production. We find strong signatures of polyphagy and detoxification in gene families associated with feeding on different hosts and in new gene families acquired by lateral gene transfer. Deep transcriptome analysis of mites feeding on different plants shows for the first time how this pest responds to a changing host environment. The compact T. urticae genome has unique attributes among arthropod genomes with remarkable instances of gene gains and losses. The completion of the T. urticae genome sequence opens new avenues for understanding the fundamentals of plant-herbivore interactions, developing novel pest management strategies and producing new bionanomaterials.

Keywords:*genomics, plant-pest interaction, genome structure, chelicerate,*

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Regulation of immune genes in silkmoths: From transcriptome to individual genes

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The innate immune system defends all metazoans against invading microorganisms. Such a defense mechanism is reported to be well conserved and relies on intricate host-pathogen interaction between pathogen recognition receptors in the host and pathogen associated molecular patterns in microbes. Drosophila and silkworm have been attractive model organisms for the study of immune system due to their well-established genetics, the absence of acquired immune system, and the remarkable correlation of their immune signaling pathways with the mammalian innate immune defense system. One of the hallmarks of the insect defense is systemic response, which involves the synthesis of small cationic antimicrobial peptides by the fat body, a functional equivalent of the mammalian liver. Two distinct and reasonably well-studied signaling pathways, the immune deficiency (Imd) and the Toll pathways, control the transcription of the antimicrobial peptide genes. Fungal or Gram-positive bacterial infections activate the Toll pathway whereas Gram-negative bacterial infection activates the Imd pathway. With the availability of whole genome sequences and RNA interference tool for various insects, many novel immune genes have been identified and their functions have been determined. In the silkmoths, through analysis of immune transcriptome, we have identified and functionally characterized a number of novel immune genes that are involved in nodulation, clearance of Gram-positive bacteria and bacterial growth arrest. Also, we have demonstrated that immune genes are sex-biased in insects. These results would add to further understanding of immune mechanisms in insects and also provide clues to look for similar mammalian proteins whose function is not understood.

Keywords:*transcriptome, silkworm, imd, toll*

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S501M09

Genetics, Genomics and Evolutionary Entomology

Sequencing small and large insect genomes

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We have sequenced the genomes of three Hymenoptera, and are in the process of sequencing three more, as well as a fly, moth, and beetle. This sequencing is being performed entirely with ILLUMINA with assembly using SOAPdenovo. The three species completed are the dwarf honey bee Apis florea, the common eastern bumble bee in the USA, Bombus impatiens, and the imported alfalfa leafcutter bee, Megachile rotundata. Each of these was performed largely from a single haploid male to avoid polymorphism and resulted in high quality draft genome assemblies of 300-500 Mbp with scaffold N50s in the 500-1000Mbp range. They are currently being annotated and analyzed. The ongoing projects on Hymenoptera are the wheat stem sawfly, Cephus cinctus, and two parasitoid wasps, Microplitis molitor and Diachasma alloeum. Again each is largely being done from single males, but using multiple males and females for mate pairs. The moth genome is that of the navel orangeworm, Amyelois transitella, the fly is the apple maggot, Rhagoletis pomonella, and the beetle is the western corn rootworm, Diabrotica virgifera. Each of these last three is based on inbred populations to reduce polymorphism. The latter two are particularly large genomes, estimated at 1 and 2.6 Gbp, respectively, and are providing a challenge to this approach to relatively cheap insect genome sequencing.

Keywords:*genome sequencing, Apid florea, Bombus impatiens, Megachile rotundata, Cephus cinctus, Microplitis demolitor, Diachasma alloeum, Amyelois transitella, Rhagoletis pomonella, Diabrotica virgifera*

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S501M10

Genetics, Genomics and Evolutionary Entomology

Molecular evolution in social insects

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The evolution of sociality represented one of the major transition points in evolutionary history. We are interested in understanding how evolutionary processes affect social systems and how sociality, in turn, affects the course of evolution. The main subjects of our research are the social insects, which display the most advanced levels of sociality and dominate ecological communities because of their sophisticated group behaviors. Our recent research has focused on understanding the molecular underpinnings of social behavior using comparative and functional genomic approaches. I will discuss studies aimed at deciphering how epigenetic inheritance affects the formation of social insect castes. In addition, I will present information on how gene evolution is coupled to phenotypic variation in insect societies. Overall, our research helps link molecular processes to the evolution of advanced societies and phenotypic diversity.

Keywords:*social insect, molecular evolution, genomics, epigenetics, Hymenoptera*

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S501M11

Genetics, Genomics and Evolutionary Entomology

Identification and analysis of conserved elements in social insect genomes

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Conserved elements or conserved regions in a genome are the genomic sequences that are highly similar across different species. The sequence conservation in these regions implies they are probably functional elements. As more and more social insect genomes have been sequenced, it is an unprecedented chance to investigate the genomic conservation across different social insect taxa. Based on the multiple whole genome alignments, we use computational methods to identify genomic regions conserved in all sequenced social insect genomes, as well as those conserved in specific groups of social insect genomes (e.g., ant group, bee group, etc). As many of the conserved elements are located in non-coding/unannotated regions, we utilize the available biological databases to annotate these conserved elements. After annotation, we try to identify the functional categories over-represented in the conserved regions and investigate their possible roles in social evolution. This study contributes to the annotation of social insect genomic sequences and sheds light on the conserved regulatory mechanisms of social insects.

Keywords:*conserved elements, social insect, comparative genomics*

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S501M12

Genetics, Genomics and Evolutionary Entomology

Comparative Genomics of Ants

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Comparative genomics is a powerful tool to identify functional genomic regions. This includes both coding and conserved non-coding DNA. Additionally, it allows inferring the origin and evolution of novel genes and changes in the amount and distribution of regulatory elements. With the publication of seven ant genomes we are in a unique position to determine genomic changes associated with the convergent evolution of eusociality (bees and ants) and compare evolutionary trends in 11 hymenopteran Genomes with that in 15 Dipteran genomes and 30 arthropod genomes, respectively. Surprisingly, the annotations for all seven ant genomes harbor a huge number of lineage specific genes and the number of lineage specific genes is negatively correlated with phylogenetic distance. Many of those lineage specific genes have expression support and seem to be functional rather than annotation artifacts. I will also discuss the evolution of gene regulation in respect to DNA methylation, conserved and transcribed intergenic RNA's, and transcription factors and transcription factor binding sites.

Keywords:*Comparative Genomics*

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S501M13

Genetics, Genomics and Evolutionary Entomology

Identification and characterization of long noncoding RNA in the Locust *Locusta migratoria* reveals roles in phenotypic plasticity regulation

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Many organisms, including insects, express phenotypic plasticity. The regulatory mechanisms underlying phenotypic plasticity are largely unknown so far. Here we reported one type of RNA molecular, long nonconding RNA (lncRNA), possess such attributes by which to trigger regulation of plasticity in a timely manner because lncRNAs do not need to translate into protein before functioning. A few dozen lncRNAs are known to play important regulatory roles in diverse processes, such as imprinting, epigenetic regulation, and development. In this study on the migratory locust Locusta migratoria, we first developed a new prediction method to identify noncoding RNA with high accuracy and sensitivity (both >91%). Based on EST and RNA-seq transcriptome data, we totally discovered 9406 distinct lncRNA expressed in different tissues and developmental stages. 41% of the lncRNAs are located within intergenic region, and 19% within intronic region. lncRNAs demonstrated high tempo-spatial specificity in expression. We then investigated lncRNAs' role in phase change regulation of the locust. Among these lncRNAs, 859 lncRNAs differentially expressed between solitary and gregarious phases of the locusts. Some lncRNAs exhibit contrasting expression pattern during time-course of isolating and crowding of locust population, imply lncRNAs' role in regulation of phenotypic plasticity.

Keywords:*long noncoding RNA, Locusta migratoria, phenotypic plasticity, phase change, aggregation*

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S501M14

Genetics, Genomics and Evolutionary Entomology

Genomic consequences of multiple speciation processes in a stick insect

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Diverse geographic modes and mechanisms of speciation are known, and individual speciation genes have now been identified. Despite this progress, genome wide outcomes of different evolutionary processes during speciation are less understood. Here, I present an integrative analysis of genomic divergence among populations of Timema cristinae stick insects undergoing ecological speciation. The result stem from a combination of whole genome sequencing, genetic linkage and association mapping, ecological and spatial information, mating trials, manipulative field experiments and analysis of 86,130 SNPs distributed across the genome. I report patterns consistent with effects of numerous factors on genomic divergence, including geographic distance, gene flow, divergence in host-plant use and climate, and selection against maladaptive hybridization (i.e., reinforcement). For example, the number of highly differentiated 'outlier loci', allele-frequency clines, and the overall distribution of genomic differentiation were recognizably affected by these factors. The results demonstrate how genomic data can provide new insights into speciation and how genomic divergence can be complex, yet predictable.

Keywords:*speciation, natural selection, population genomics*

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S502M01

Genetics, Genomics and Evolutionary Entomology

Transposon structure and function in multiple eukaryotic systems

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Transposons play a significant role in insect genetics and biotechnology. Insect whole genome projects have revealed the transposon landscape in several insect genomes with some variance in both transposon load and in the numbers of superfamilies present within a species. Despite the larger numbers of transposons present in insects, only six are commonly used for the genetic manipulation of insects, these being P and hobo (virtually exclusively in Drosophila melanogaster in which strains lacking active P and hobo transposons were established several decades ago), piggyBac, Mos1, Minos and Hermes, with piggyBac proving to be the most robust transposon not only in insects but in some protozoa and in some vertebrates as well where, in humans, it is being developed as a gene therapy vector. All these transposons were found in insects, most were discovered through experiments that detected their mobility, and all were found before the advent of whole genome projects. Without question their use in insect genetics and biotechnology has had a great impact on these fields, however we still know little about how endogenous and foreign transposons are regulated in their host cells and we have yet to thoroughly mine insect genomes for new transposons which may have mobility properties different or even superior to the ones now in use. These investigations are facilitated by high throughput sequencing approaches which may enable us, at least in descriptive terms initially, to describe the genomic background in which transposons find themselves both in their original host genome and in new

Keywords:*Eukaryotic Systems*

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S502M02

Genetics, Genomics and Evolutionary Entomology

Transposon-based genetic analysis in *Anopheles stephensi*

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Advanced transposon-based genetic technologies are now available for use in Anopheles mosquitoes that provide new opportunities for performing forward and reverse genetic analyses leading to new advances in our understanding of Anopheles gene structure and function. Transposons can configured to carry specific sensors that permit the detection of various genetic elements such as enhancers, promoters and genes. Here I report that the piggyBac transposon in Anopheles stephensi is highly active after initial integration into the genome and can be used in combination with the Gal4/UAS system to efficiently detect enhancers and genes. On average, 1.3% of the progeny arising from enhancer-trap screens harbored an enhancer-detection event. A collection of lines with patterns of Gal4 expression of particular use to vector biologists has been assembled and will be described. In addition, a functional gene-trap system was created based on a promoter-less reporter gene with a 3' splice-acceptor site. This system is functional and being used to conduct forward genetic analyses of salivary gland- and midgut-function in adult females. The availability of these powerful functional genomics technologies in conjunction with currently available structural genomic resources will enhance efforts to investigate the genetic basis of important aspects of mosquito biology. These technologies also provide the vector biology community with new genetic resources.

Keywords: *Transposon-based*

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S502M03

Genetics, Genomics and Evolutionary Entomology

Transgenic silkworms for analysis of gene function and production of recombinant proteins

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The complete genomic sequence of the silkworm Bombyx mori was published firstly in lepidopteran insects. The assembly of 432 Mb genome is a reference in the genomic analysis of the very diverse order of Lepidoptera. In post-genomic studies, various genetic tools such as binary expression systems, enhancer traps, gene traps, insertional mutagenesis, gene targeting, and RNAi are useful for analysis of gene function. To develop the versatile genetic tools for various insects is very important. We have been developing the genetic tools using transgenic silkworms for gene functional analysis and new uses. We established the method to generate the transgenic silkworm using piggyBac and Minos transposons. We have constructed some new marker genes. Then we developed the binary expression systems in the silkworm using a GAL4/UAS or Tet-On/Off system. Also we have constructed the piggyBac-based GAL4 enhancer trap and gene trap systems by remobilizing transposon vectors to various chromosomal locations. Such systems can be used for targeted transgene expression in a stage- and organ/tissue-specific manner, and insertion of the transgene may disrupt the endogenous gene, creating a new mutant allele. Several enhancer trap lines were successfully used to determine the function of classical mutants. Recently, we have been developing a gene targeting system using ZFNs and TALENs, in addition to a PhiC31 integrase system. We have also constructed an efficient system for producing recombinant proteins/silks using a transgenic technology and silk-synthesis system of silkworm in the silk gland.

Keywords:*transgenic silkworm*

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S502M04

Genetics, Genomics and Evolutionary Entomology S502

A new hAT-family transposon vector, hopper, from the Oriental fruit fly, *Bactrocera dorsalis*

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The hopper hAT-family transposable element isolated from the oriental fruit fly, Bactrocera dorsalis, is distantly related to both the Drosophila hobo element and the Ac element from maize. The original 3,120 bp element was highly degenerate and appeared to have a mutated transposase, while a second 3,131 bp element isolated from a white eye strain had a transposase reading frame and terminal sequences consistent with function. This putative functional element was used to create a binary vector:helper transformation system by linking the transposase to a D. melanogaster hsp70 promoter, and creating vectors marked with the D. melanogaster mini-white+ gene and polyubiquitin-regulated DsRed markers. Both vectors were used to transform D. melanogaster, and the DsRed vector was used to transform the Caribbean fruit fly, Anastrepha suspensa, indicating a wide range of function in dipteran species. However, transformation frequencies were approximately 5% per fertile G0, which is relatively low compared to other widely used systems for Drosophila and non-drosophilid species.

Keywords:*transposable element, hAT-family, Bactrocera dorsalis, germ-line transformation*

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S502M05

Genetics, Genomics and Evolutionary Entomology

Site-specific recombination for transgene targeting in tephritid genomes

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Insect transgenesis is mainly based on the random integration of DNA fragments embedded into non-autonomous transposable elements (e.g. piggyBac, mariner, Minos, etc.). Once a random insertion into a specific location of the genome has been identified as particularly useful in respect to transgene expression, ability to make the insertion homozygous, and lack of fitness costs, one would like to take advantage of that location for further modification and improvement. Efficient methods for the modification of previously inserted transgenes are site-specific integration systems like the attP-attB/ phiC31 system. Such a system can be used to exchange and/or stabilize transgenes in the respective tephritid genome. This will help to combine several transgene-encoded advantageous traits at evaluated genomic positions to generate optimized strains for environment-friendly pest control. Advances in site-specific integration systems in tephritids are shown.

Keywords:*programmed cell death, hid, Sterile Insect Technique, homologous recombination*

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S502M06

Genetics, Genomics and Evolutionary Entomology

Genetic control of vector-borne pathogens

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Genetic strategies have been proposed to control the transmission of vector-borne pathogens. Approached that seek both population suppression and population replacement of mosquitoes have benefitted from the development of transgenesis, site-specific recombination and targeted effector molecules. Advances in the development of vector-based genetic control strategies for preventing malaria parasite and dengue virus transmission have resulted in tools that in laboratory trials either suppress completely mosquito populations or render them incapable of transmitting the pathogens. Field trails are in progress for some population suppression strains, while the population replacement approaches still require the development of safe and efficacious gene-drive systems.

Keywords:*mosquito transgenesis*

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Disrupting mosquito attraction to host cues by targeted mutagenesis of the orco olfactory co-receptor

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Human host odor is a long-range, attractive cue that guides mosquitoes to their hosts. The mosquito perceives differences in host odor, both between and within species, to determine which host to feed upon. The molecular mechanism by which mosquitoes translate host odor information into host-seeking behavior has been inferred but not demonstrated. Insects use two families of olfactory receptors, the Ionotropic Receptor (IRs) and the Olfactory Receptors (ORs), to detect volatile chemicals in their environment. To shed new light on mosquito olfactory-driven behaviors, we developed a technique for targeted mutagenesis in Aedes aegypti using zinc-finger nucleases (ZFNs) and proved that it can work by heritably disrupting GFP. In addition, ZFNs can induce homology dependent repair allowing for genetic modification at a specific site. To understand the role of ORs in mosquito behavior, we targeted orco, a gene formerly known as Or83b in Drosophila and Or7 in mosquitoes, which is an obligate co-receptor for ORs. We demonstrate that Aedes orco is necessary for electrophysiological responses to 1-octen-3-ol but not to CO2. We are currently using olfactometer-based assays to understand how orco null mutant mosquitoes respond to human and non-human host odors, chemical repellents, and plant volatiles. The establishment of loss-of-function genetics in Aedes aegypti will open new paths of investigation in the genetics of vector biology.

Keywords:*mosquito, zinc-finger nuclease, olfaction, chemical repellents, host-seeking*

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S502M08

Genetics, Genomics and Evolutionary Entomology S502

Development of 'male-only' strains of the New World screwworm fly, *Cochliomyia hominivorax*

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The New World screwworm (NWS) fly, Cochliomyia hominivorax, is a serious pest of warm-blooded animals. The Sterile Insect Technique (SIT) was used to eradicate the NWS from U.S.A., Mexico and Central America. Currently, the USDA-APHIS and the Panamanian government jointly operate a NWS mass-rearing and sterilization facility in Pacora, Panama. Sterilized male and female NWS are released continually in a 'buffer zone' in Southern Panama to prevent re-introduction from South America. APHIS has identified several advantages to incorporating transgenic NWS strains into the control program. These include strains that carry stable fluorescent proteins for monitoring released flies and strains that facilitate the release of only males, which would increase the efficiency of SIT. We are currently developing transgenic NWS strains marked with fluorescent proteins that carry tetracycline-repressible female lethal genetic systems. To achieve sex-specific gene expression we have isolated the transformer (tra) gene from NWS. A conditional female-lethal gene cassette was made consisting of the tetracycline transactivator (tTA) gene interrupted by the NWS tra intron and driven by a minimal promoter with multiple tTA binding sites. Alphey and colleagues have previously shown that a similar auto-regulated tTA gene is lethal to female fruit flies raised without tetracycline (inhibits tTA). Transgenic NWS, Lucilia cuprina and Drosophila melanogaster lines have been made carrying the female-lethal gene cassettes and are currently being bred to homozygosity. Various characteristics of the lines (e.g. female viability, fecundity, longevity, male competitiveness) will be investigated.

Keywords:*RIDL, SIT, screwworm, Lucilia*

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S502M09

Genetics, Genomics and Evolutionary Entomology

A transgene-based maternally-driven RNAi system produces male-only progeny in the Mediterranean fruit fly, *Ceratitis capitata*

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In Ceratitis capitata, female sex is determined by Cctransformer (Cctra), which engages during XX embryogenesis a positive feedback loop by maternally provided CcTRA and secures the continuous production of CcTRA in XX developing individuals. In Ceratitis XY embryos, a Y-linked M factor promotes, either directly or indirectly, the inclusion of additional sequences into the Cctra mRNAs which contain in-frame stop codons that truncate the CcTRA ORF, causing the XY embryos to develop into adult males. Likewise, transgene in vivo silencing of Cctra by RNAi causes a breakdown of the positive feedback loop in XX embryos leading them to develop as fertile XX males. One of our a dozen transgenic lines line displayed an unexpected maternal effect. Transgenic females crossed with non transgenic males give rise to male-only XX and XY progeny irrespective of whether they carry the transgene. The observation that Cctra is functionally switched OFF even in non-transgenic XX siblings, suggests a maternal effect of the transgene. In the transgenic mothers RNAi could deplete eggs of maternal CcTRA protein that is required for zygotic activation of Cctra or maternally transmitted dsRNA molecules may be responsible for repressing early activation of zygotic Cctra. To further investigate the molecular nature of this maternal effect we are currently examining the genomic sequences surrounding the insertion site, which possibly drive transgene-expression in the female germ line. These findings may help to design a repressible transgene-based maternally-driven RNAi system which can be used as a novel sexing strategy to produce male-only progeny for SIT.

Keywords:*sex determination, RNA interference, sexing, Sterile Insect technique*

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S502M10

Genetics, Genomics and Evolutionary Entomology

Transgenic approach in the genetic control of Lepidopteran insects, *Bombyx mori* and *Ostrinia furnacalis*

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The sterile insect technique (SIT) is an environmentally friendly, species-specific genetic control method, which largely depends on advancement of the insect transgenic technique. SIT or RIDL (release of insects carrying a dominant lethal) has been applied successfully in pest management such as the Mediterranean fruit fly, Ceratitis capitata and the yellow fever mosquito Aedes aegypti. However, there is seldom report about the genetic control of Lepidopteran insects which contains large amounts of pests and insects with economic importance. Here we present two different strategies in the genetic control of two Lepidopteran insects, the silkworm Bombyx mori and the Asian corn borer Ostrinia furnacalis. In B. mori, a male-only rearing technique used sex-specific alternative splicing has been established and showed female-specific lethality during the early larval stage. In O. furnacalis, a different strategy based on the sex chromosome-linked gene manipulation technique is undergoing to induce sex-linked lethality. A stable transgenic line of O. furnacalis has been established and this will shed light on the future genetic control of O. furnacalis.

Keywords:*genetic cntrol, trangenic technique, bombyx mori, Ostrinia furnacalis*

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Genetics, Genomics and Evolutionary Entomology

First and second generation paratransgenesis for control of vector-borne diseases

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Despite great advances in public health, insect-transmitted infectious diseases remain a leading cause of morbidity and mortality. Currently, the best methods for control of insect-borne diseases involve the use of chemical pesticides. Such campaigns may yield spectacular results, yet longterm efficacy remains a problem. Environmental toxicity, adverse effects on human health,emergence of insect resistance and the prohibitive cost limit the use of many pesticides.Therefore, the elimination of insect pests is neither practical nor probable. Evolving methods for control of vector-borne diseases rely on modification of insects. Paratransgenesis is a "Trojan Horse" approach to control of disease transmission. It employs the interactions between diseasetransmitting vectors, bacterial commensals of the vectors and transmitted pathogens. Commensal bacteria are isolated and genetically transformed in vitro to export molecules that interfere with pathogen transmission. The genetically altered bacetria are introduced into the host vector where expression of engineered molecules affects the host's ability to transmit the pathogen. This approach attempts to decrease pathogen transmission and employs, as a gene delivery mechanism, bacterial flora native to the host vector. The model system for paratransgenic control involves the vectors of Chagas disease, a disease of Central and South America. Paratransgenic methods are under development for control of sand fly-mediated leishmaniasis and Pierce's Disease, an agricultural disease of grapes and citrus crops that is transmitted by Glassy Winged Sharpshooters. Finally, second generation paratransgenic systems are under development that employ advanced nano-materials to achieve better targeting of recombinant molecules with minimal environmental impact of transgene release. This session will

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Paratransgenesis to control the transmission of malaria

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Malaria is a devastating disease that infects about 500 million people a year and kills between 1 and 2 million, mostly African children. About half the human population is at risk of infection. Additional strategies to complement vector control by insecticides and parasite control by drugs are urgently needed. One such alternative is paratransgenesis, the indirect alteration of the phenotype of the vector by the introduction of transgenic microorganisms into the midgut lumen. The E. coli hemolysin A secretion system was used in the mosquito symbiotic bacterial species, Pantoea agglomerans, to deliver a variety of anti-Plasmodium effector proteins in Anopheles sp. Engineered strains of P. agglomerans were able to inhibit the development of Plasmodium falciparum (a human parasite) and P. berghei (a rodent parasite) by up to 98%. Importantly, the fraction of mosquitoes carrying any oocysts at all (=prevalence) was decreased by up to 84%. We will also discuss efforts to move this system into other bacterial symbionts like Asaia sp. which are reported to colonize other mosquito tissues and display vertical transmission within mosquito populations. This work provides the basis for the use of paratransgenesis to complement other strategies to reduce the burden of malaria on human populations.

Keywords:*paratransgenesis, malaria*

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Genetics, Genomics and Evolutionary Entomology

Panarthropod phylogeny based on mitochondrial genomes

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The phylogenetic positions of the phylum Tardigrada and the class Pycnogonida (Euarthropoda, Chelicerata) have been considered one of the most controversial issues in metazoan phylogeny. Up to date, the controversies still remain unclear although a variety of molecular data such as EST, mitochondrial genomes, and hox genes have been adopted for resolving the enigmatic problems. Here, we have done intensive phylogenetic analyses based on mitochondrial genomes of 4 tardigrades including the two new ones, a eutardigrade Pseudobiotus spinifer and a heterotardigrade Batillipes longispinosus, and 7 pycnogonids recently published. The gene arrangements of P. spinifer and B. longispinosus are identical to that of Limulus polyphemus except for the placement of tRNAIle (I) between tRNALeu(CUN) (L1) and tRNALeu(UUR) (L2) and peculiar placement of control region (CR). The present mitochondrial proteome phylogeny joins Tardigrada and Pycnogonida with Acariformes within Chelicerata sensu lato. Tardigrada, Pycnogonida, and Acariformes may have a minute rudimentary abdomen, a stylet or stylet-like mouthpart for piercing, and loss of a respiratory organ (except for some mites). Such types of morphological characters may arise with the evolution of microscopic body size and/or aquatic life. It implies that aquatic lives shown in water bears, sea spiders, and water mites may result from one or more aquatic invasion event(s) of certain terrestrial arachnids. Unjointed legs in tardigrades might be gained through secondary loss from jointed legs during the adaptive evolution of a certain terrestrial chelicerate in aquatic environment.

Keywords:*Tardigrada, Pycnogoida, Panarthropod phylogeny, Mitochondrial genomes*

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Genetics, Genomics and Evolutionary Entomology

Structure, evolution and phylogeny of polyneopteran mitogenomes

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Polyneoptera is an assemblage of 11 insect orders consisting of lower neopteran insects. The interordinal relationships within Polyneoptera remain highly controversial and ancient rapid radiations are thought to be responsible. Only the monophyly of Dictyoptera and the sister relationship of Grylloblattodea and Mantophasmatodea have been repeatedly recovered by various molecular and morphological studies. Phasmatodea, whose phylogenetic position among Polyneoptera is quite unstable, has been thought to be a key taxon to resolve the polyneopteran phylogeny. Embioptera is the preferred candidate of the sister group of Phasmatodea but the lack of sequenced mitochondrial genome from this order prevented the molecular phylogenetic analysis based on mitogenome. We have determined the almost complete mitochondrial DNA (mtDNA) sequence of an Embioptera species (Aposthonia japonica) and explored the phylogeny of polyneopteran insects using nearly complete mitogenome sequences. Our analyses recovered a close relationship between Phasmatodea, Mantophasmatodea and Grylloblattodea. Dictyoptera was placed as a sister to this group. The monophyly of Orthoptera was confirmed and Plecoptera was placed as a sister group of this order. A. japonica was placed as sister to Verophasmatodea, a suborder of typical stick and leaf insects. There is a translocation of a large region including atp6, atp8, cox3, nad3, and nad5, as well as a duplication of the 12S rRNA gene.

Keywords:*compositional bias, translocation, molecular phylogeny, Embioptera*

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Genetics, Genomics and Evolutionary Entomology

Phylogenomic, transcriptional and evolutionary studies of mitochondrial genomes for orthopteran insects

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The complete mitochondrial genomes of 50 species from Orthoptera were determined using primer walking with cloning sequencing strategy after Long PCR amplification. These mitochondrial genomes were comprehensively analyzed in properties with respects of gene content and rearrangement, base composition, codon usage, tRNAs and rRNA secondary structures, and structual domains in A+T-rich region. The phylogenetic relationships at taxonomic levels of subfamilies, families and superfamilies of Orthoptera were reconstructed based on complete mitochondrial genome data, from which we can obtain some important conclusions about the phylogentic relationships among major higher catogories of Orthoptera insects. Based on a robust phylogenetic tree, the evolutionary constraints of mitochondrial genome with respects of flying capacity (according to wing type) and ecotypes (according to habitats) were tested. The results shown that Ka/Ks is statistically significant different between longwinged and wingless insects, but no significant different among ecotypes. Finally, the mitochondrial genome transcriptional map of Oxya chinese were constructed based on the data from total RNA of larvae and adult samples through de novo transcriptome sequencing.

Keywords:*Orthoptera, mitochondrial genomes*

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What can mitochondrial genomes tell about beetle evolution?: Strengths and pitfalls of mitochondrial phylogenomics

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The ability to generate large molecular datasets for phylogenetic studies is a boon to biologists, but such expansion of data also introduces numerous analytical problems. Mitochondrial phylogenomics, the use of complete mitochondrial genomes (mtgenomes) in phylogenetics, provides a manageable platform for address various challenges in analyzing phylogenomic data. In this presentation, I discuss both strengths and pitfalls of analyzing mtgenome data in the context of the mitochondrial genome phylogeny of the insect order Coleoptera (Arthropoda: Insecta). Beetles comprise one of the largest radiations in the Tree of Life, containing more than 350,000 species, or approximately 25% of all described species. As a part of an NSF-funded Beetle Tree of Life Project, a large number of complete mtgenome sequences of major lineages within Coleoptera have been generated. I present the most comprehensive beetle mitochondrial phylogenomic study to date and discuss the resulting patterns, especially with respect to overcoming systematic bias in phylogenomic data.

Keywords:*Coleoptera, mitochondrial genomics, phylogenomics*

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Mitogenomics of the Coleoptera under dense taxon sampling

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This study exploits next-generation sequencing technology for sampling of ~250 mitochondrial genomes from major lineages of the Coleoptera. The resulting tree resolves basal relationships with good support, and provides an increasingly reliable estimate of phylogenetic relationships within Coleoptera at the family level. We show that, if taxon sampling is sufficiently dense, full mitochondrial genomes are very strong markers in insect phylogenetics at the intra-ordinal level, despite the sometimes disappointing results of whole mitogenomes at inter-ordinal level and the difficulty of resolving deep relationships with individual mitochondrial genes. Yet, a few taxa show highly divergent mitogenomes, and cannot be placed reliably due to apparent long-branch attraction.

Keywords:*Mitogenomes, Coleoptera, next-generation sequencing, phylogeny*

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Genetics, Genomics and Evolutionary Entomology

Mitochondrial genome evolution in Hymenoptera

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During the last 10 years, we have been investigating the utility of mitochondrial genome sequence data for investigating relationships within the highly diverse insect order Hymenoptera. We have been interested in two sources of phylogenetic information; (i) gene position and (ii) aligned DNA sequence data. However, prior to using gene position to infer phylogeny, we wished to establish whether gene position is selectively neutral, and whether all gene rearrangements are equally likely. We investigated the evolution of 67 tRNA mitochondrial gene rearrangements and found that gene position was generally selectively neutral. However, not all genes appear to be as mobile as others. For example, genes close to the AT-rich region are marginally more mobile than others, while some tRNA genes appear to move rarely. We also investigated the recovery of phylogeny using mitochondrial genome sequence data from 29 hymenopteran taxa, when aligned DNA sequences from each of the 13 protein-coding genes and 2 rRNA genes are analysed. We find that the Bayesian analysis of 1st and 2nd codon position nucleotide data to be superior than any other type of analysis. However, we consistently find that terminal branch lengths are very long relative to internal branch lengths. This suggests that a much higher level of taxonomic sampling is required than is currently available, if these data are to be used to address long-standing phylogenetic uncertainties in the Hymenoptera.

Keywords:*gene rearrangement, tRNA, bayesian analysis, Hymenoptera*

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Phylogenetic position of Hesperiidae in Lepidoptera using mitochondrial genome data with the description of newly sequenced skippers

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Lepidoptera is one of the largest insect orders, but the phylogenetic relationships within this order, have yet to be adequately described. One of the unresolved relationships includes the monophyly of the Papilionoidea in relationship with the monotypic superfamily Hesperioidea. We present four new hesperid mitochondrial genomes (mitogenomes), representing three subfamilies: Pyrginae (Daimio tethys and Erynnis montanu), Coeliadinae (Choaspes benjaminii), and Heteropterinae (Carterocephalus silvicola). Along with the newly sequenced hesperid genomes phylogenetic analysis was conducted with all available lepidopteran mitogenomes, consisted of ~50 species in seven lepidopteran superfamilies. The test for the effect of optimization schemes (exclusion and inclusion of third codon position of 13 PCGs, 22 tRNAs, and two rRNAs, with and without partitions) also was performed. Currently, the datasets are under phylogenetic analysis, so resultant phylogenetic relationships of Lepidoptera including the position of Hesperiidae will be updated as further comprehensive analyses is completed.

Keywords:*Lepidoptera, Hesperioidea, phylogenetic relationships, mitochondrial genome*

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Insect mitochondrial genome database (IMGD): An integrated platform for insect mitochondrial genomes

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Mitochondrial genomes offer markers useful for understanding evolutionary relationships among insects. In 2009, the Insect Mitochondrial Genome Database (IMGD) was developed to support phylogenetic studies using insect mitochondrial genome sequences and comparison of genomic features across species. During the last three years, many researches have been conducted with the IMGD. Currently, the IMGD archive fully 255 sequenced mitochondrial genomes and 342,013 sequences in partially sequenced mitochondrial genomes from 74,896 hexapod species. In this database, through the species-driven user interface, users can quickly select and analyze mitochondrial genomes at any taxonomic levels with eight analysis functions. Moreover, species taxonomic analysis and distance matrix analysis recently introduced provides in-depth dissections. The IMGD has been a versatile informatics platform and will be maintained systematically for conducting systematic phylogenetic and comparative analyses of insects.

Keywords:*IMGD, mitochondrial genome, insect, phylogenetic analyses*

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Regulation of transposons in pestiferous mosquitoes and tephritid fruit flies that are subjects of SIT programs

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The genome-wide regulation of transposons in at least Drosophila melanogaster has been proposed to occur at least, in part, by small RNAs which, in a dicer-independent pathway. We were interested to determine if these piRNAs also exist in the mosquito Aedes aegypti and, if so, whether they are specific to the large and diverse transposon complement of this insect pest. We also sought to determine whether the expansion of genes involved in piRNA biogenesis in Aedes led to any of these genes having different stage and tissue specific expression relative to their orthologs in D. melanogaster. We find that while many piRNAs in Aedes are targeted to transposons, many also target protein-coding genes. We also find that some of the piRNA biogenesis genes of Ae. aegypti are expressed in somatic tissues. The implications of these findings will be discussed.

Keywords:*pestiferous mosquitoes*

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Transgenic sexing system for *Ceratitis capitata* (Diptera: Tephritidae) based on female-specific embryonic lethality

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The Sterile Insect Technique (SIT) is more efficient and cost-effective when only sterile males are released. A female-specific lethality system based on a female-specifically spliced intron had been developed for transgenic sexing in theMediterranean fruit fly Ceratitis capitata to possibly overcome the fitness problems of classical genetic sexing strains. However, this transgenic sexing system showed lethality predominantly during the pupal stage. To create an early-acting lethality system, we combined the principle of this female-specific lethality system with an embryonic lethality system to yield an early-acting sex separation system. This female-specific embryonic lethality system makes use of a sex-specifically spliced intron that was artificially introduced into a hyperactive pro-apoptotic gene. The expression of this pro-papoptotic gene is driven by a conditional heterologous transactivator that is itself under the control of a blastoderm-specific promoter. Different transgenic combination strains showed varying strengths of female-specific embryonic lethality with some lines showing 100% female-lethality at embryonic stages while the lethality does not affect male flies which eclose at normal rates and behave regularly. Transgene modification by site-specific recombination in the sexing effector line was further performed and subsequent stabilization of the transgene by transposase-induced partial removal achieved to make this line safer for applications in operational SIT programs.

Keywords:*apoptosis, binary expression system, cellularization genes, differential splicing, sex determination*

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Transcriptional profiles of mating responsive genes from testes and male accessory glands of the Mediterranean fruit fly, *Ceratitis capitata*

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Insect seminal fluid is a complex mixture of proteins, carbohydrates and lipids, produced in the male reproductive tract. This seminal fluid is transferred together with the spermatozoa during mating and induces post-mating responses in the female. Molecular characterization of seminal fluid proteins in the Mediterranean fruit fly, Ceratitis capitata, is limited, although studies suggest that some of these proteins are biologically active. We report on the functional annotation of 5914 high quality expressed sequence tags (ESTs) from the testes and male accessory glands, to identify transcripts encoding putative secreted peptides that might elicit post-mating responses in females. The ESTs were assembled into 3344 contigs, of which over 33% produced no hits against the nr database, and thus may represent novel medfly sequences. Extraction of the coding sequences resulted in a total of 3371 putative peptides. The annotated dataset is available as a hyperlinked spreadsheet. Four hundred peptides were identified with putative secretory activity, including odorant binding proteins, protease inhibitor domain-containing peptides, antigen 5 proteins, mucins, and immunity-related sequences. Quantitative RT-PCR-based expression analyses of a subset of putative secretory protein-encoding transcripts from accessory glands indicated changes after one or more copulations when compared to virgin males of the same age. Our results suggest that synthesis of the putative secreted proteins that play an active role in the seminal fluid may be transcriptionally regulated. This represents the first large-scale dataset for novel studies on functions and processes associated with the reproductive biology of Ceratitis capitata.

Keywords:*Ceratitis capitata*

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Female-specific embryonic lethality strains for the Mexican fruit fly, *Anastrepha ludens*

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Transgenic strains were created for reproductive sterility and female-specific lethality to improve the biological control of tephritid fruit fly pests in the Anastrepha genus, based on a tetracycline-suppressible (Tet-Off) system to conditionally regulate the embryonic expression of a pro-apoptotic cell death gene. The Tet-off system consists of a driver cassette having the A. suspensa embryo-specific serendipity α gene promoter linked to a Tet-transactivator (tTA) used to drive expression of a phospho-mutated variant of the pro-apoptotic cell death gene, hid, from A. ludens. The AlhidAla2 variant was validated as having high cell death activity in in vitro A. suspensa cell death assays, that also determined the most efficient driver-lethal effector cassette combinations for use in Anastrepha transformants. This resulted in two hybrid transgenic A. suspensa strains exhibiting 100% lethality on tet-free diet, one being 96% lethal in embryos with no survival past the first larval instar, which is critical for pests that are most damaging in late larval stages. For female-specific lethality necessary for male-only strains, the Assryα-tTA embryonic driver strain was used with a new lethal effector strain (TREhs43-Cctral-AlhidAla2) having a medfly transformer intron placed in the 5' end of the AlhidAla2 lethal effector. The medfly tra intron is sex-specifically spliced, which removes translational stop codons from the female reading frame, and not the males, resulting in female-specific AlhidAla2 lethal expression. Creating driver-female-specific lethal effector hybrid strains in A. suspensa resulted in two strains having double-heterozygous transgene cassettes being 100% male-only, while four male-only strains were created having double-homozygous cassettes.

Keywords:*embryonic lethality, female-specific lethality, cell death genes, tet-suppresible lethality, sterile insect technique*

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Assessment of genetic diversity of the olive fly Israeli SIT laboratory strain

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Early attempts to apply the SIT on the olive fly, Bactrocera oleae, were unsuccessful, mainly due to the low competitiveness of the sterile mass-reared males compared to the wild ones. Recently, new efforts are underway in Israel to develop a vigorous and efficient mass-reared olive fly laboratory strain. In order to understand the genetics of the adaptation process and possibly link the corresponding loss of competitiveness to genetic markers, we followed the fluctuation of genotypic frequencies of ten microsatellite markers during the course of 22 generations of the colonization of a wild Israeli population in laboratory conditions. Effective and observed allele number is halved after 11 generations, so is mean heterozygocity. Practically, there is very little change between F0 and F1, there are substantial changes between F1 and F2-F5, and there is a virtual complete adaptation to the new laboratory environment by F11, since no more changes are observed between F11 and F22. If we assume that the loss of allele number and heterozygocity also reflects the loss of the "wild" character of the colonized strain and, possibly, the loss of a substantial part of its natural vigor, our results indicate that there is an apparent need to enrich a mass-reared colony at least every five to eight generations. Furthermore, simulation models indicated that while most of the observed frequency fluctuations were due to random drift, some alleles were probably under selection.

Keywords:*olive fly, adaptation, laboratory conditions, selection, drift*

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Towards the development of improved sterile insect release protocols: Genome analysis of three Australian tephritid pests

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We have sequenced the genome and transcriptome of three native Australian tephritids, including Bactrocera tryoni, the Queensland Fruit Fly, which is the major pest of horticulture in Australia and, increasingly, in the South Pacific. The other two species, B. neohumeralis and B. jarvisi, are also pests of agriculture in Northern Australia, but show different host fruit preference, dispersal ability, level of adaptation to laboratory and factory conditions, morphological differentiation and mating behaviour. Never-the-less, we are able to hybridise all three species in the laboratory. This will enable us to investigate the DNA correlates of important between-species differences. The B. tryoni genome has been sequenced to 60 times coverage and assembled with a current N50 of 16kb although many of the scaffolds are greater than 1Mb. The assembled scaffolds contain mainly unique sequences. Although partial, near-complete and complete sequences for several families of transposable elements are abundant, these generally do not interfere with assembly. The Maker annotation software predicted over 10,000 transcripts based on both the transcriptomic data and de novo predictions. Analysis of the data will identify the full range of transposable element families within each species. This will be of use in predicting the compatibility of new transposable element constructs for future transformation experiments. In addition, degraded elements can provide unique genetic species markers. Comparison of sex determination genes in all three species will allow RNAi constructs with conserved tra or tra-2 gene sequences to be developed for construction of male-only SIT strains.

Keywords:*Bactrocera, genome sequencing*

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Development and characterization of genetic sexing strains of the oriental fruit fly, *Bactrocera dorsalis*, and the guava fruit fly, *Bactrocera correcta* for SIT

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The oriental fruit fly, Bactrocera dorsalis (Hendel), and the guava fruit fly, Bactrocera correcta (Bazzi), are key economic pests for commodity fruits in Asia and Pacific regions. Area-wide Integrated Pest Management programmes using Sterile Insect Technique have been implemented in order to suppress fruit fly populations in several pilot areas in Thailand. Sterile insect techniques (SIT) continues to reveal areas where new technologies can improve the effectiveness of fruit fly control. One SIT advancement concerns genetic improvement of insect strains. Two stable white pupae-based genetic sexing strains have been developed and maintained. They were B. dorsalis and B. correcta and named Salaya1 and Salaya2, respectively. Mass rearing and release conditions for the Salaya1 have been evaluated. A modular mass-rearing facility for the sexing strain has been constructed and used to produce flies for open field trial, resulting in suppression of the wild population and lower fruit infestation rate. Moreover, microsatellite DNA makers have been characterized, evaluated and applied to monitor the quality of the mass reared strain and used for strain identification. These markers have also been used to study re-mating in a field cage test.

Keywords:*Tephritidae, oriental fruit fly, Bactrocera dorsalis, microsatellites, genetic sexing strain*

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Approaching problems for Anastrepha fraterculus SIT with genetic and molecular tools: reproductive strategy, sex determination, and loss of variability in the lab

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In South America Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) is an important fruit pest, sharing habitat in some subtropical and temperate regions with Ceratitis capitata (Wiedemann). Both species cause economical losses on fruit producing areas, and limit their access to international markets due to quarantine restrictions imposed by the fruit-fly-free countries. Currently, only insecticides are used to reduce the density of wild populations of A. fraterculus, so the use of environmental friendly control strategies against this pest is strongly recommended. We are performing laboratory studies on the biology, behaviour and genetics of these two species focusing on helping to the integrated actions against this pest and providing useful information about rearing and genetics to the development of SIT technique. Recently we participated in the isolation and characterization of A. fraterculus microsatellite markers, an achievement that opened a wide door to the study of the genetic variability of wild populations of this pest and, to the knowledge of the biological and physiological changes suffered by these insects when they are introduced to artificial rearing, thus understanding the genetic processes underlying during the adaptation to laboratory conditions. Our initial results revealed that microsatellite markers both in laboratory and wild populations of A. fraterculus from Argentina are highly polymorphic for 15 markers analyzed so far, whereas other 10 markers are under evaluation. A number of studies are in progress in order to use this molecular tool to solve biological problems as reproductive strategy, sex determination, loss of variability in the lab.

Keywords:*reproductive strategy, sex determination, and loss of variability in the lab*

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Development and evaluation of genetic sexing strains of *Anastrepha ludens* for sterile insect technique

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A genetic sexing strain (GSS) has been developed for the Mexican fruit fly Anastrepha ludens based on a Y-autosome translocation system. In this GSS typically males emerge from brown (wild type) and females from black (mutant) pupae. Cytogenetic analysis of mitotic chromosomes of this strain showed a reciprocal translocation between the longest chromosome 2 and the Y chromosome. Polytene chromosome analyses showed significant ectopic pairing of chromosome element III and therefore suggest that this chromosome is involved in the translocation and therefore corresponds to chromosome 2 of the mitotic complement. This GSS strain has been named "Tapachula-7" and was scaled to semi-mass rearing in the operational Moscafrut Program. A filter rearing system is implemented to maintain the integrity of the strain. , i.e. each generation aberrant flies are removed from the mother stock according to their pupal colour phenotype. The egg hatch has been around 70%, which is a value higher than theoretically expected for a strain carrying a Y-autosome translocation. The evaluation of the fitness and genetic stability in a semi-mass rearing system has been carried out at densities of 3000 pairs of flies per cage each generation; quality control parameters were measured in order to establish reference parameters of the filter rearing system. The genetic studies of aberrant individuals (crosses with "black pupae" mutants and "wild type" flies) help us to identify possible events that could destroy the genetic sexing system.

Keywords:*Anastrepha ludens, Mutants, Genetic Sexing System, Cytogenitic*

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Improving the sterile sperm ID method for its implementation in the aw-SIT program against *C. capitata* in Spain

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In area-wide SIT programs against Ceratitis capitata, the success of the released sterile males is regularly measured by indirect methods, considering that the SIT program works when the ratio is balanced to sterile males. However, this method does not take into account the real target of the SIT, which is the wild female. To fulfil this gap, a sperm ID method based on molecular sperm identification in trapped females was developed in 2007 for the Spanish medfly populations. This protocol required three working days to analyse only 30 samples, which made difficult its practical application for aw-SIT programs due to the high handling time and personnel cost. The objectives of this work were: 1) to check of the validity of the sperm ID method with medflies from different world regions, and 2) to improve it and adapt it to hightroughput techniques for its cost-effective application to aw-SIT programs. Firstly, we validatedthe developed PCR markers. All the tested wild populations lacked the HaeIII characteristic haplotype of the sterile male lines, rendering the markers universal for other regions. Secondly, by modification of the spermathecae extirpation procedure and their conservation protocol,we were able to reduce handling time and costs (more than 96 samples analysed per run). In addition, this new protocol is able to detect sterile sperm in preserved wild female's samples trapped under field conditions for more than 28 days. The implementation of this improved sperm ID method to an aw-SIT program as the one in course in Eastern Spain is discussed.

Keywords:*medfly, PCR-RFLP, strain diagnosis, hightroughput technique*

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S504TU11

Genetics, Genomics and Evolutionary Entomology

Field evaluation of a pupal color genetic sexing strain of the mexican fruit fly, *Anastrepha ludens*

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A genetic sexing strain (GSS) of the Mexican fruit fly, Anastrepha ludens, was developed in 2008 in Mexico and has been recently evaluated for potential use in SIT programs in Mexico and the USA. The pupal color strain,Tapachula-7, is based on a recessive black pupae color gene in which the wild-type normal allele for brown color is genetically linked to the male Y-chromosome by means of a Y-autosome chromosomal translocation. Female pupae, black in color, and male pupae, normal brown in color, can be separated using electronic sorting machines to produce large populations of virtually all-male pupae for release in the field. A population of the strain was moved to Guatemala's fruit fly facilities for further larger scale rearing, quality control testing, and field evaluation. Since November, 2011 a team of investigators from Mexico, Guatemala, and the USA has been carrying out a series of field studies comparing both the GSS and a standard bisexual strain against wild A. ludens, including: field cage mating competition and outdoor survival in Retalhuleu, Guatemala; induced pupal suppression in large field cages in Antigua, Guatemala; and fly dispersal and longevity in the open field in south Texas, USA. As of March, 2012, the GSS has been of superior quality compared to the standard bisexual strain and very competitive with wild flies. Barring an unforeseen problem in the final stages of field testing, the GSS should soon provide a high quality, improved replacement for the current bisexual strain of A. ludens in future SIT programs.

Keywords:*Anastrepha ludens, genetic sexing strain (GSS), field cage mating competitiveness, C-values, field s*

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Development of genetic sexing strains in the codling moth

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SIT programmes for population control of the codling moth, Cydia pomonella, are based on bisexual releases. Male-only releases have never been tested in the field due to the lack of efficient sexing method. We proposed to develop genetic sexing lines in this pest by inserting a selectable transgene (a dominant conditional lethal mutation) into the female-specific W chromosome. Under restrictive conditions the transgene would abort development of females and thus, non-transgenic male-only offspring would be produced for irradiation and release. For germ line transformation of the codling moth, we have prepared a new plasmid construct called RATom. The RATom is based on a piggy-Bac vector and contains a red fluorescence marker gene, tdTomato, a partial sequence of the codling moth ortholog of the Drosophila Notch gene (CpNotch), the 3xP3 promoter, and gypsy insulators. The truncated CpNotch is expected to enable the intracellular Notch function at room temperature, while blocking embryonic development of transformed insects at a lower temperature. Functionality of the construct was verified in transfected cells of the embryonic Bombyx mori cell line BM1. Currently, its function is examined using a USA-Gal4 system in Drosophila lines transformed with RATom-pUAST construct to optimize temperature for conditional lethality. We also started with transgenic experiments in the codling moth. Moreover, we constructed a gene-based physical map of the codling moth Z chromosome using BAC-FISH. The map revealed that the codling moth Z had evolved through a translocation of an autosome, corresponding to Bombyx chromosome 15, onto the ancestral Z chromosome.

Keywords:*codling moth, genetic sexing, sex chromosomes, SIT, transgenesis*

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S504TU13

Genetics, Genomics and Evolutionary Entomology

Role of the Y chromosome in the *Ceratitis capitata* sex determination and reproduction.

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In Ceratitis capitata the sex determination occurs very early during embryogenesis: a Y-linked M factor (still to be isolated) represses the expression of a key master epigenetic switch, transformer (Cctraep) which promotes female sex determination,. In XX embryos, Cctraep is ON, and positively autoregulates throughout development. Transient or in vivo RNAi repressing Cctraep leads to the production of XX and XY male flies. Interestingly, XX males are fertile, indicating the absence of relevant Y-linked fertility factors. XX males transfer efficiently GFP-marked sperms into females. In a small scale competitive mating experiments, wild type females did not show any preference toward wild type or XX males. The microscopic observations of female storage organs revealed motile sperm cells in almost all the females mated either with wild type or with XX males. Experiments to assess the morphology and the ultrastructure of the reproductive apparatus as well as the protein pattern of the accessory glands from XX males are in progress. Experimental release of XX transgenic males and XY wild type males in semi-field conditions are underway to assess their competitiveness and evaluate their potential applications in the Sterile Insect Technique programs. We approached the identification of the Y-linked M factor(s) by subtraction approaches: mRNA samples, extracted from XX-only embryos and from mixed XX-XY embryos, were analyzed by Illumina mRNAseq and in silico subtraction. The initial results have led to the identification of dozens of putative male-specific transcripts which are under experimental validation.

Keywords:*sex determination, Y chromosome, reproduction, Sterile Insect technique, transcriptomics*

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Towards identifying sex determination pathway genes in the silkworm *Bombyx mori* for their possible use in genetic sexing strategies

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With the recent developments in genetic and molecular basis of sex determination mechanisms and insect transgenesis, precise genetic sexing tools are now available mostly in dipterans, and are beginning to be exploited in the control of agriculture insect pests and human disease vectors. Such studies are scanty in Lepidoptera which embraces a large number of pest species that inflict heavy crop damage. The silkworm, Bombyx mori, unlike hymenopterans and dipterans, has the female heterogamety (ZW), and W chromosome plays decisive role in the female sex determination. Bombyx mori doublesex (Bmdsx) gene is sex-specifically spliced to yield male-specific and female specific polypeptides. The female splicing of Bmdsx pre-mRNA represents the default mode. BmPSI (P-element somatic inhibitor) is shown to work along with BmIMP (Bombyx homolog of IGF-II mRNA binding protein) to promote male specific Bmdsx splicing by binding to Exon Splicing Silencer (ESS) of exon 4. Recently, we have discovered two CCCH-type and C2H2-type type zinc finger proteins-encoding genes that map to the minimal W chromosome region that is sufficient to confer the femaleness in the silkworm. The experimental evidence suggests that these zinc finger proteins have role in sex-specific Bmdsx splicing pattern. We have identified an additional protein Bmhrp 28 (B. mori heterogeneous nuclear ribonucleoprotein 28) binding to ESS being expressed in both the sexes and its role as splicing repressor is being elucidated. These studies have bearing on the ongoing efforts to develop genetic sexing strains in Lepidoptera on the lines of dipteran agriculture pests and human disease vectors.

Keywords:*lepidoptera, genetic sexing, bombyx*

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Cross mobility of piggyBac-like elements

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PiggyBac-like elements (PLE) are widespread in variety of organisms, however, few of them are active or have an intact transposon structure. Fortunately, we isolated two new functional PLEs, AyPLE1.1 and AgoPLE1.1 from the noctuid species Agrotis ypsilon and the cotton aphid Aphis gossypii respectively. The cross-mobility assays between the active transposases (IFP2, AyPLE1.1 and AgoPLE1.1) and the transposon ITR sequences from HvPLE1.1、TcPLE1、AgoPLE1.1、Uribo2、BmPBLE04、BmPBLE07 and BmPBLE69 were studied. The results showed neither of the functional transposase could recognize ITR sequences from other resources but its own. The results indicated ITRs determined the specificity of a functional transposase of piggyBac transposons. Similar cross-mobility assays between the active transposases (IFP2, AyPLE1.1 and AgoPLE1.1) and the sub-IR sequences from IFP2, TcPLE1 and PgPLE were studied. The results showed each of the three functional transposase could recognize all the sub-IR sequence from IFP2, TcPLE1 or PgPLE, and made precise excision events. However, statistics analysis revealed that the number of precise excision events were severely affected by alternation of different sub-IR sequences. These results show the possibility to increase the transposition frequencies of piggyBac by using its optimal sub-IR. These preliminary results were obtained in the S2 cell cultures, further studies in vivo were still needed.

Keywords:*piggyBac, cross mobility, transposon*

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The role of apyrene sperm in the polyandrous swallowtail butterflies

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Females of the swallowtail butterfly mate multiply throughout their life span. During each copulation, the male butterfly fills first a white gel, structures a spermatophore in the bursa copulatrix of the female, and then transfer sperm. Spermatophores collapse, and represent nutrients for the polyandrous females to increase their longevity as well as fecundity. On the other hand, a large spermatophore protects a male's reproductive investment by delaying female remating. Immediately after the completion of copulation, both types of sperm, eupyrene and apyrene sperm, are found in the spermatophore. The eupyrene sperm is still in bundle, and they are gradually released in the spermatophore. About 9,000 eupyrene and 265,000 apyrene spermatozoa are counted in a single copulation. Because apyrene sperm are not in bundle, apyrene sperm migrate from the spermatophore to the spermatheca earlier than eupyrene sperm. Accordingly, if females mated multiply, the time difference may avoid the mixing of sperm, due to constructing the barrier of apyrene sperm of the mated male. Therefore, the predominance of sperm from the last mating session occur not in the bursa copulatrix but in the spermatheca. Since the spermatheca remains constant in size during the female life span, however, the excess sperm migration may cause the spermatheca to overflow. The spermathecal gland leading from the distal end of spermatheca is used for gradually filling eupyrene spermatozoa of the previous male. Selected hypothesis on the role of apyrene sperm proposed will be examined in the viewpoint of sperm competition.

Keywords:*spermatophore, spermatheca, apyrene sperm, eupyrene sperm, multople mating*

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Evolution of ultrasonic courtship signals through sensory bias in moths

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Animals have evolved sexually attractive signals for successful mating. One origin of sexual communication can be attributed to the perception of predator-related cues. However, the evolutionary processes by which a receiver's sensory bias toward predator cues is coopted as a mating signal have remained enigmatic because receivers do not respond sexually to predators. Ultrasonic communication used by some moths in mating is thought to originate from the detection of ultrasonic calls emitted by bat predators, although this has not been demonstrated experimentally. Male Asian corn borer moths Ostrinia furnacalis (Lepidoptera, Crambidae), produce a 'whispering ultrasound' that immobilizes females, allowing the male to copulate freely. Here, I show that the corn borer moth male-courtship ultrasound and the ultrasound produced by bats are perceived similarly by female corn borer moths and generate a 'freezing' response in both cases. Thus, male courtship-ultrasound takes advantage of the avoidance response, making copulation easy. In contrast, both sexes of toxic Japanese lichen moths Eilema japonica (Lepidoptera, Arctiidae) generate warning clicks against attacking bats, and these females use male courtship-ultrasound as a signal for mate-recognition rather than freezing. These results support the notion that seductive mating signals have evolved under sexual selection to exploit preference by receivers that do not originally confound mate cues with predator ones.

Keywords:*Acoustic communication, Mating behavior, Receiver bias, Predator, Cognition*

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The presence of rivals reduces reproductive fitness of male moths

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Increasing evidence shows that spermatogenesis is costly. Therefore, males should optimize the use of their sperm to maximize their reproductive outputs in the dynamic natural environment. However, experimental evidence on how males do this is largely lacking. Here we examine how a male in copulation responds to the presence of rivals or additional mates and how such response influences his lifetime reproductive fitness. The model animal used here is a polyandrous moth Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) where a female receives more than enough sperm from a single mating. We show that when rival males are present around a copulating pair the male ejaculates more sperm to his mate but in long term he inseminates fewer females, sires fewer offspring and lives shorter. The opposite is the case when additional females are present during copulation. These findings suggest that males can adjust ejaculate size in response to the risk of sperm competition and opportunity for remating. When the risk of sperm competition is high, the male ejaculates more sperm to his mate probably because the relative number of competing sperm in a female from different males determines the outcome of their relative paternity. However, the elevated ejaculation expenditure shortens the male longevity and reduces the number of females he can inseminate, resulting in fewer offspring sired by him in his lifetime. When the risk of sperm competition is low and the opportunity to mate with more females is high, males save sperm to inseminate more mates and sire more offspring.

Keywords:*Sperm competition, Ephestia kuehniella, ejaculate, sex ratio*

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Comparative evidence that copulatory spermatophore guarding is associated with smaller nuptial gifts and modifications in the form and/or use of the male's cerci in bushcrickets.

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The optimal mating duration, and/or volume of ejaculate transferred per mating, is expected to differ between the sexes, with male optima often exceeding those of females. Male crickets and bushcrickets exhibit a range of behaviours, including nuptial feeding (the spermatophylax) and copulatory spermatophore guarding (CSG), that appear to function to deter the female from interfering with ejaculate transfer. The hypothesis that these traits are analogous in function predicts that, across taxa, relative spermatophylax size will be reduced in species with CSG. The aims of this study were to test this prediction and to examine qualitatively differences in the structure and use of the male's cerci (which are used to grasp the female) between species with and without CSG. Data on ejaculate mass, spermatophylax mass and the duration of copulation following spermatophore attachment were obtained for 40 species of Tettigoniid. As predicted, relative nuptial gift mass correlated negatively with the duration of CSG across species. Males of species with CSG showed modifications in the structure and/or the use of the males' cerci, apparently increasing their effectiveness as grasping devices. Such adaptations included: elongated cerci which encompass the end of the female's abdomen; cerci with an increased number of projections and cerci in which the cercal teeth puncture the female's cuticle. The distribution of CSG within the Tettigoniidae suggests that this behaviour has evolved independently several times within the family.

Keywords:*Spermatophore guarding, genitalia, spermatophylax, sexual conflict, nuptial feeding*

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S505TU05

Genetics, Genomics and Evolutionary Entomology

What's so interesting about spider sperm?

Marie E Herberstein
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Most models of sperm competition assume mobile sperm that can actively compete with each other over fertilisation. Spider sperm offer a very different perspective as sperm tails are rolled up during transfer and require activation within the female sperm storage organ to gain mobility for fertilisation. This scenario places unique selective pressures on sperm evolution and opportunities for female control. In this presentation I will unveil the impressive, but possibly underestimated potential that spiders offer in the field of sexual selection and sperm competition.

Keywords:*Araneae, haplogyne, entelegyne*

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Should inbreeding avoid or tolerate? Inbreeding avoidance or tolerance? Comparison of mating behavior between strong- and weak- inbreeding depression strains of the sweet potato weevil

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Inbreeding depression is the decline of fitness as a result of mating with kin individuals. When inbreeding depression occurs, mating with a close relative is often considered maladaptive. However, in some situations, breeding with close relatives can provide inclusive fitness benefits and the benefits outweigh the cost of inbreeding. The sweet potato weevil Cylas formicarius has been mass reared over 74 generations using the sterile insect technique. Strains of the weevil exhibit varying extents of inbreeding depression (strong inbreeding depression: s-strain; and weak inbreeding depression: w-strain). Theory predicts that s-strain weevils will avoid inbreeding and w-strain weevils will tolerate inbreeding. To examine this prediction, we compared the effect of relatedness on the mating success of the two strains. Close-relative pairs of the w-strain copulated less frequently than non-kin pairs, but almost all s-strain pairs copulated — irrespective of relatedness. These results showed that the strain with weak inbreeding depression avoided inbreeding, and strain with strong inbreeding depression tolerated inbreeding. The contradiction between the theoretical prediction and our results is discussed from mating system and life history of the sweet potato weevil.

Keywords:*domestication, inbreeding depression, kin selection*

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Nuptial feeding diversity and its morphological basis in scorpionflies (Mecoptera: Panorpidae)

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Scorpionflies have interesting courtship and copulatory behaviors of nuptial feeding, which have been confirmed to have a morphological basis. The males with short notal organ have developed salivary glands. During courtship the male secretes a salivary secretion(s) as nuptial gift. Males of Neopanorpa longiprocessa have a well-developed notal organ, but have simple salivary glands and do not secrete salivary masses. The male usually uses his notal organ to hold the female for 1?2 h without nuptial gifts, likely a forced copulation. Males of Panorpa liui have complex clamp-like notal organ and undeveloped salivary glands, only providing dead insect as nuptial gift, without salivary masses. Males of Dicerapanorpa magna "dance" while disseminating sex pheromone. After a female is attracted to nearby, short-period copulation (20?60 min) was observed without nuptial feeding; then the male secretes a salivary mass, which is consumed by the female during long period of copulation (1?3 h). The male sometimes offers additional salivary secretions to prolong copulation. After the salivary secretion(s) has been consumed out, the copulation often elongate to extra 34?90 min without feeding. To sum up, remarkable diversity of courtship and copulation behaviors of scorpionflies is intimately correlated with their notal organs and salivary glands. Developed notal organ is associated with simple salivary glands, and vice versa. Nuptial gifts reduce sexual conflict by increasing paternity investment and supplying nutrients to females. This finding may play a role in the study of behaviour evolution and phylogeny of Panorpidae.

Keywords:*Panorpidae, courtship, copulation, salivary glands, nuptial gift*

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Scramble competition and sperm competition in a sexually cannibalistic mantis

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In species where males do not actively fight for access to females, competition between males largely consists of a race to locate their mates (scramble competition) and to fertilise their eggs (sperm competition). Scramble competition polygyny is perhaps the most common mating system in insects, and yet the factors influencing its evolution are largely unstudied. The sexually cannibalistic praying mantis, Pseudomantis albofimbriata exhibits a polygynous mating system, and females are highly cannibalistic, consuming approximately 40% of males encountered. In this species, both scramble competition and sperm competition therefore interact with the cannibalistic nature of females to determine the optimal mating strategy for males. Here, I will describe some recent research on P. albofimbriata demonstrating (a) last-male sperm precedence and (b) greater attraction to unmated females. I also show that when males are kept under conditions of high perceived risk of competition, they (b) develop to maturity more slowly, and (d) transfer larger ejaculates. Together, these results show that different components of male competition can interact in intriguing ways to determine the strategies that males use to maximize reproductive success.

Keywords:*Mantodea, Scramble competition, Sexual Cannibalism*

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S505TU09

Genetics, Genomics and Evolutionary Entomology

Sexual selection on *Toxotrypana curvicauda* Gerstaecker (Diptera: tephritidae)

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The papaya fruit fly, Toxotrypana curvicauda (Diptera: Tephritidae) is important to Mexican economy and is distributed in America from Florida to South America. Its chemical ecology and some aspects of the biology and behavior were revised previously; however, most information regarding sexual selection is missing. Female age triggers courtship and mating. Only 6-d-old females or older were proceptive and receptive. In non-choice and double choice tests, male age was not relevant to achieve mating. Male repertory and courtship vigor were similar at all ages tested. In one-choice tests, males that had already achieved one mating showed a weaker courtship than virgin insects. Mated males (24 or 48 in advance) invested less time in courtship than virgins' even in presence of mated or virgin females. Courtship and mating patterns (orientation, approaching, corporal movements, contact and mating) had different frequency in virgin that in previously mated couples. In double-choice laboratory experiment, nor males or females selected its partner based on weight, however, males always selected virgin females for mating while females may mated indistinctly with virgin or mated males. Finally, male hierarchy defined the male achieving mating.

Keywords:*mate selection, gonadic maturation, courtship*

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Male responses to sperm competition: Adaptation, or are males just paranoid?

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Males in many taxa are known to exhibit behavioural plasticity in response to the perceived intensity of sperm competition This is reflected in the fruit fly Drosophila melanogaster by increased copulation duration following prior exposure to a rival. We tested the prediction that males do not adult their copulation effort in response to the presence of rivals in Drosophila species where there is little or no sperm competition. Contrary to expectations, male plasticity in copulation duration was found in all species, even those where females rarely remate. The only exception was Drosophila bifasciata - in this species males transfer a mating plug to females during copulation. Our results will be discussed in relation to the adaptive basis of plasticity in these species.

Keywords:*Sperm competition, Sexual selection, Drosophila*

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Intra-specific variation in the effect of male-derived substances on female mating behaviours in *Callosobruchus chinensis*

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I demonstrated the intra-specific variations of the effect of male seminal substances influencing female oviposition, longevity, and fecundity among strains of the adzuki bean beetle, Callosobruchus chinensis. Previously, the effect of male seminal substances that induced female oviposition and reduce female longevity was identified. In this study, I examined the differences in the effect of male substances and female response to based on female oviposition, longevity, and fecundity among four strains of the C. chinensis with different re-mating frequency (mC, 5.9%: jC-S, 8.8%: akC02, 29.8%: isC, 38.4%). With respect to oviposition and longevity, the effect of male substances, female response to them, and interaction between male and female among strains were affected. Thus, female strains did not respond consistently to male substances from male strains extracted. Furthermore, female fecundity was also affected the effect of male substances, female response to them, and interaction between male and female among strains. These results indicate the variation and the possibility of evolutionary interaction of reproductive traits that affect reproductive success between both sexes.

Keywords:*co-evolution, induce oviposition, male-derived substances, male toxic substances, female fecundi*

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S506TU01

Genetics, Genomics and Evolutionary Entomology

Post-blood feeding changes in the transcriptomes of vector mosquitoes

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Hematophagy is a physiological trait ancestral to all extant mosquito lineages. We hypothesize that classes of genes were selected early in the evolution of mosquitoes to facilitate efficient blood-feeding and that orthologues of these genes are shared among modern mosquitoes. Furthermore, we propose that the mechanisms of transcriptional regulation of these genes may have been conserved along with the functional components and be reflected in conservation of cis-regulatory elements and modules (CREs and CRMs) in the control DNA. We are using transcriptomic analyses of genes expressed specifically in the midguts of four species, Aedes aegypti, Anopheles gambiae, Anopheles stephensi and Culex quinquefasciatus (Diptera: Culicidae), representing two subfamilies (Culicinae and Anophelinae) estimated to have diverged 145-200 million years ago, to identify and cluster genes. These data will leverage a combined comparative-genomics and expression-profile based CRE/CRM discovery strategy. The discovered CRMs will serve as the basis for validation of a set of conserved CREs that may be combined to drive robust anti-pathogen effector gene transcription in the midguts of vector mosquitoes directly following the ingestion of a bloodmeal.

Keywords:*hematophagy midgut*

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S506TU02

Genetics, Genomics and Evolutionary Entomology

Exploiting different host plants: A spider mite genomic and transcriptome perspective

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The two-spotted spider mite Tetranychus urticae is an important crop pest and one of the most striking examples of polyphagy among arthropod herbivores. Recently the whole genome of this mite species was sequenced and reported. This genome is the first among chelicerates, and of great importance for general comparative phylogeny. It is also the first sequenced genome of a highly polyghagous crop pest and offers a unique opportunity to search for genomic and evolutionary signs of polyphagy and xenobiotic metabolism in arthropods. We annotated and studied known gene families implicated in detoxification, and found a species-specific proliferation of genes that might be related to remarkable ability of spider mites to survive on so many host plants. To investigate the role of these gene families in spider mite polyphagy, we used RNA-seq and microarray based transcriptome profiling of mites feeding on different hosts. We show that the expression patterns of mites feeding on different hosts are often correlated, defining a set of genes and gene families for further study. This genome-wide unbiased approach confirmed the important role of 'the usual suspects' in xenobiotic metabolism, but also uncovered several new gene families with a putative role in detoxification and stress response. We studied some of these gene families in detail, and reveal how they were acquired by horizontal transfer. Our results sheds new light on the remarkable adaptive potential of this crop pest and strongly argue that the xenobiotic metabolism of spider mites needs to be studied beyond classical detoxification genes.

Keywords:*xenobiotic metabolism, detoxification, polyphagy*

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S506TU03

Genetics, Genomics and Evolutionary Entomology

Deconstruction of the plant primary cell wall: An insect perspective

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The primary plant cell wall is composed of an association between several polysaccharides, proteins and lignin. These polysaccharides include cellulose, hemicelluloses and pectins which represent the most abundant polysaccharides on the planet and a rich source of energy. Microorganisms, especially plant pathogens, are very efficient in utilizing these polysaccharides because their genomes possess genes encoding a range of enzymes able to degrade them. In contrast, the presence of such genes in higher eukaryotes is rare. The association between termites and their symbiotic protists is a text book example of cellulose digestion in insects, where not only the protists provide cellulose-degrading enzymes but also the insect itself. Recently, we demonstrated through transcriptome analyses, that the genomes of phytophagous beetles, especially species from the superfamilies Curculionoidea and Chrysomeloidea, possess diverse and large multi-gene families encoding a range of plant cell wall degrading enzymes (PCWDEs). To characterize these gene families and their respective function, we established an insect/plant model, the Mustard leaf beetle Phaedon cochleariae/Brassica rapa. I will present the results we got from the analysis of the larval gut lumen proteome of this beetle (I) to determine whether there are enzymes able to degrade plant primary cell wall polysaccharides and (II) to identify the proteins responsible for the enzymatic activities. We also compare the results obtained from this proteomics approach to those obtained from the analysis of the beetle transcriptome. I will also present a detailed functional characterization of one of the gene families that popped up after our combined proteomics/transcriptomics approach.

Keywords:*phytophagous beetles, plant primary cell wall, proteomics, transcriptomics, horizontal gene transfer*

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Molecular organization of *Drosophila* gustatory receptors in enteroendocrine cells

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The gastrointestinal tract is emerging as a major site of chemosensation in mammalian studies. Enteroendocrine cells are chemosensory cells in the gut which produce regulatory peptides in response to luminal contents to regulate gut physiology, food intake, and glucose homeostasis, among other possible functions. Increasing evidence shows that mammalian taste receptors and taste signaling molecules are expressed in enteroendocrine cells in the gut. Invertebrate models such as Drosophila can provide a simple and genetically tractable system to study the chemosensory functions of enteroendocrine cells in vivo. To establish Drosophila enteroendocrine cells as a model for studying gut chemosensation, we used the GAL4/UAS system to examine the expression of all 68 Gustatory receptors (Grs) in the intestine. We find that 12 Gr-GAL4 drivers label subsets of enteroendocrine cells in the midgut, and examine colocalization of these drivers with the regulatory peptides neuropeptide F (NPF), locustatachykinin (LTK), and diuretic hormone 31 (DH31). RT-PCR analysis provides additional evidence for the presence of Gr transcripts in the gut. Our results suggest that the Drosophila Grs have chemosensory roles in the intestine to regulate physiological functions such as food uptake, nutrient absorption, or sugar homeostasis.

Keywords:*gustatory receptor, enteroendocrine cell, regulatory peptide, Drosophila*

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Midgut transcriptomics of an invasive insect species

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The insect midgut represents one of the major tissue interfaces that deal with several important physiological functions including digestion, detoxification and immune response. The emerald ash borer (Agrilus planipennis), is an exotic invasive insect pest that has killed millions of ash trees (Fraxinus spp.) primarily in the Midwestern United States and Ontario, Canada. However, despite its high impact status little knowledge exists for A. planipennis at the molecular level. An initial pass using Roche-454 pyrosequencing resulted in 25,173 high quality expressed sequence tags (ESTs) for larval A. planipennis midgut. A high number of the midgut sequences contained chitin-binding peritrophin (248) and trypsin (98) domains. Further, the midgut transcriptome of A. planipennis revealed putative microbial transcripts encoding for cell-wall degrading enzymes such as polygalacturonases and endoglucanases. A significant number of SNPs (137) and microsatellite loci (317) were also predicted in these sequences. qPCR quantification of cytochrome P450s belonging to various CYP clades revealed distinct expression patterns at the tissue level. On-going RNA-Seq experiments i.e. differential gene expression in A. planipennis larvae reared on resistant versus susceptible ash trees and small RNA profiling should provide new insights into the midgut physiology of this invasive insect pest. These findings will lay the foundation for future functional studies in A. planipennis and the results obtained will be essential both at the applied and basic scales.

Keywords:*midgut, Agrilus planipennis, ESTs, gene expression, invasive insect*

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Transcriptomic responses of the lepidopteran midgut to xenobiotics

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The fall armyworm Spodoptera frugiperda is a major pest of agriculture that is highly polyphagous, feeding on more than 25 host plant families. This phytophagous insect has thus developed sophisticated mechanisms to metabolically inactivate some of the potentially toxic xenobiotics that it encounters. To investigate patterns of gene induction in response to xenobiotics, we have contructed a Spodoptera frugiperda microarray consisting of 9773 60-mers oligonucleotides designed from EST libraries obtained from eight different tissues, developmental stages and treated insects. We addressed this array with cDNAs prepared from midguts of larvae fed with 5 plant allelochemicals, 4 insecticides and 2 model inducers, as well as from Sf9 cells treated with those compounds. We show that each chemical has a quite specific response, with few genes affected in common between all treatments. This implies that there are multiple transduction pathways interacting to provide the insect with an adapted response to its chemical environment. The response of Sf9 cells has little in common with the in vivo response of midgut tissue. We also studied by RT-PCR the response of various CYP genes to the eleven chemicals and will report on the catalytic activity of inducible P450s that are produced in a yeast heterologous system.

Keywords:*transcriptome, xenobiotics, pesticides, midgut, Sf9 cells, Spodoptera frugiperda*

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Genetics, Genomics and Evolutionary Entomology

CD36 family proteins are responsible for the tissue specific uptake of certain carotenoid species in the silkworm, *Bombyx mori*

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The silkworm, Bombyx mori provides very clear examples of tissue-specific delivery for certain carotenoid species from the midgut lumen to the silk gland via hemolymph lipoprotein, lipophorin. Lipophorin contains lutein and beta-carotene as a complex mixture of lipids in the hydrophobic core. The yellow (C) or flesh color cocoon (F) mutants accumulate lutein or beta-carotene in the middle silk gland from lipophorin, respectively. We have reported that the C gene encodes Cameo2, a member of CD36 gene family which has been known to function as as lutein specific transporter and transmembrane non-internalizing lipophorin receptor in the middle silk gland. The nature of the F gene was defined by positional cloning and transgenic rescue experiments with the binary GAL4-UAS expression system. The F gene encodes SCRB15, a paralog of Cameo2 with 26% amino acid identity. SCRB15 may be responsible for beta-carotene specific non-internalizing lipophorin receptor. In the F mutant, SCRB15 is not expressed because the mRNA structure of SCRB15 is severely disrupted due to a 1.4 kb insertion in a coding exon. This finding that both C and F genes encode CD36 homologs raises questions how CD36-homolog proteins recoginize the difference of carotenoid species which permits to uptake the middle silk gland from lipophorin.

Keywords:*CD36, carotenoid, lipophorin*

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S506TU08

Genetics, Genomics and Evolutionary Entomology

Physiological genomics of lepidopteran larval digestive lipases

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A major change in lipid quality occured with the evolution of autotrophy with galactolipids becoming the dominant source of long chain unsaturated fatty acids. Adapting to these novel lipids may have been yet another challenge in the evolution of phytophagy, in particular phyllophagy. Transciptome analysis of lepidopteran larvae midgut sequences identified lipases similar to GPLRP2, a vertebrate digestive enzyme with activity against galactolipids. We have measured galactolipase, phospholipase and triacylglycerol lipase activities in the midgut of six lepidopteran larval species, two folivores, two granivores, a carnivore and a keratinophage. The ratio of galactolipase to triacylglycerol lipase was high in the two folivores and low in the four other species, matching the major lipids in thier respective diets. In order to understand the evolution of galactolipase activity we have studied the exon-intron structure of insect digestive lipases and compared them with those from vertebrates of the same lipase superfamily.

Keywords:*galactolipase*

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Midgut enzymes from the spruce budworm: A genome-scale investigation

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Midgut enzymes are the frontline proteins involved in the conversion of insect food into insect biomass, and they have attracted significant interest for their potential as targets for insect control. While midgut enzymes can be characterized by directed protein or RNA sequencing from midgut tissue (proteomics, RNAseq, etc.), whole genome sequencing can provide a valuable complementary approach for the discovery of midgut enzymes. We are currently completing the sequencing and assembly of the genome of the spruce budworm (Choristoneura fumiferana) one of the most serious pest of fir and spruce in Canada. Genome sequencing was performed by a combination 454 GS FLX sequencing of BAC libraries and shotgun sequencing as well as GAIIx Illumina sequencing. Assembly has generated approximately 28 000 scaffolds covering 75% of the C. fumiferana genome. We will present results of the annotation of the major groups of digestive enzymes, e.g. peptidases, glucosidases, lipases and other proteins assisting in digestion. This genome-scale study will allow the characterization of unique patterns of digestive enzyme gene structure and function in a conifer-feeding Lepidoptera.

Keywords:*Digestion, enzyme, lepidoptera, genome, spruce budworm, Choristoneura fumiferana*

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S507W01

Genetics, Genomics and Evolutionary Entomology

Networks: Small non-coding RNAs write the screenplay

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The abstract concerns the introductive talk of the Symposium on "Small non-coding RNAs (sncRNAs) and genome expression". A huge amount of data is available for the description of sncRNAs in insect genomes as well as their role in regulating mRNAs or transposons. The sncRNAs usually act through physical interactions with their targets (mRNA, transposons) and the interactions may in some cases affect, reorient the fate of a phenotype. I will suggest the urgent need of federating approaches between entomologists and genomicists in one hand, and bioinformaticians and mathematical modelers in the other hand, in order to tackle from a pan-genomics perspective the whole regulatory networks required for biological adaption processes of insect to their environment and during their development. Examples will be taken from our studies on aphids and phenotypic plasticity. These networks are/will be a first step of a further integration of data, knowing that post-transcriptional regulation mediated by sncRNA is tightly linked to transcriptional (via transcription factors) and epigenetics (via chromatin modifications).

Keywords:*miRNAs, piRNAs, siRNAs*

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The role of microRNAs in host-pathogen interactions

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MicroRNAs (miRNAs) are small (~22 nucleotides) non-coding RNAs that play significant roles in various biological processes, including development, differentiation, apoptosis and immunity. In addition, pathogen or cellular encoded miRNAs play important roles in host-pathogen interactions by targeting the pathogen-encoded genes directly or by altering the expression of host genes that are beneficial to the pathogen. In turn, pathogens may manipulate their host miRNAs to facilitate their replication or enter into latency phase. Currently, there is little known about the role of miRNAs in insect host-pathogen interactions. Comparison of infected versus non-infected hosts using microarray or deep sequencing approaches provides valuable information with regard to identification of differentially expressed host miRNAs upon infection. In addition, pathogen-encoded miRNAs or small RNAs generated in host cells can be identified. We are using a number of host-pathogen systems to gain a better understanding of their role in the interactions. An overview of the latest outcomes in this area will be presented and discussed.

Keywords:*microRNA, host, pathogen, virus*

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S507W03

Genetics, Genomics and Evolutionary Entomology

Non-coding RNAs in *Bombyx mori*

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Accumulating evidences show that small non-protein coding RNAs (ncRNAs) play important roles in development, stress response and other cellular processes. The silkworm is an important model for studies on insect genetics and control of lepidopterous pests. Here, we have performed the first systematic identification and analysis of intermediate size ncRNAs (50?500?nt) in the silkworm. We identified 189 novel ncRNAs, including 141 snoRNAs, six snRNAs, three tRNAs, one SRP and 38 unclassified ncRNAs. Forty ncRNAs showed significantly altered expression during silkworm development or across specific stage transitions. Genomic comparisons revealed that 123 of these ncRNAs are potentially silkworm-specific. Analysis of the genomic organization of the ncRNA loci showed that 32.62% of the novel snoRNA loci are intergenic, and that all the intronic snoRNAs follow the pattern of one-snoRNA-per-intron. Target site analysis predicted a total of 95 2′-O-methylation and pseudouridylation modification sites of rRNAs, snRNAs and tRNAs. We further employ Chip-Q-PCR to detect the functional role of some ncRNAs, and our results suggested that at least two ncRNAs act by engagement in epigenetic pathway. Together, these findings provide new clues for future functional study of ncRNA during insect development and evolution.

Keywords:*Silkworm, Non-coding RNA, Development*

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Gender and stressor specific microRNA expression in *Tribolium castaneum*

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MicroRNAs (miRNAs) are small non-coding RNAs mediating post-transcriptional regulation of gene expression in eukaryotes. Addressing their role in regulation of physiological adaptations to environmental stress in insects we selected the red flour beetle Tribolium castaneum as a model. Beetles were fed with the bacterial entomopathogen Pseudomonas entomophila (to mimic natural infection), injected with peptidoglycan (experimental setting of strong immune responses) or subjected to either mild heat shock or starvation. Differential expression of selected immunity and stress-related genes was quantified using real-time PCR and expression and induction of 455 mature arthropod miRNAs was determined using proprietary microarrays. We found that Tribolium exhibits both gender- and stressor-specific adjustment of immune gene and miRNA expression. Strikingly, we discovered that the number of stressor induced miRNAs in females is remarkably higher than in males. This observation could support the hypothesis called Bateman´s principle in immunity that predicts gender specific immune responses because females gain fitness through increased longevity, whereas males gain fitness by increasing mating rates. Our results suggest that Tribolium males and females display differential regulatory elements, both pre- and post-transcriptional, likely resulting from different investment strategies in life history traits (Freitak et al. 2012: Biology Letters, in press).

Keywords:*Tribolium castaneum, microRNA, gender studies, epigenetic, immunity, environmental stress,*

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The roles of miRNAs in metamorphosis development in *Chilo suppressalis* (Walker)

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To understand the roles of microRNA in the metamorphosis development, we first constructed small RNA library from four stages of rice stem borer, Chilo suppressalis. We obtained 57 conserved miRNAs by homology searching and 91 novel miRNAs by de novo identification. We tested all 148 miRNA using microarray in seven development points, ageing larvae (aL), prepupa (pP), early pupa (eP), compound eye (cE), pretarsus (pT), pupa elongation (Pe) and adults (Ad). As results, 76 miRNAs were successfully detected in these samples. Among which, 18 of them are differently expressed between seven tested-development stages. Two novel miRNAs, csu-1 and csu-2, show the highest expression at stage aL and cE, respectively. The csu-miR-277 showed about more than 10 times change between stage pP,cE and Ad, suggesting that csu-miR-277 play an important role in metamorphosis. Csu-miR-275a was highly expressed in pP, which may also participate in PCD and tissue lysis. Csu-miR-34 shows low expression change from pP to Pe. These results proved that microRNA have key roles in insect metamorphosis and might be used as the targets of pest control.

Keywords:*miRNA, C. suppressalis (Walker) , metamorphosis, pest management*

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S508W01

Genetics, Genomics and Evolutionary Entomology

Genetic architecture of sexually selected traits in acoustic Lepidoptera

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Models sexual selection predict linkage disequilibria between genes influencing male traits and female preferences, owing to non-random mate choice or physical linkage. Such linkage disequilibria can accelerate the evolution of traits and preferences to exaggerated levels. Theory and empirical findings on species recognition suggest that such linkage disequilibria may result from physical linkage, but this possibility has seldom been addressed within the context of sexual selection. We studied the genetic architecture of sexually selected traits by analyzing signals and preferences in an acoustic moth, Achroia grisella, in which males attract females with a train of ultrasound pulses and females prefer loud songs and a fast pulse rhythm. Both male signal characters and female preferences are heritable traits. We employed a standard crossing design between inbred lines and used AFLP markers to build a linkage map for this species and locate quantitative trait loci (QTL) that influence male song and female preference. Our analyses revealed QTLs of moderate strength that influence various male signal and female receiver traits, and one QTL exerting a major influence on the pulse-pair rate of male song. However, we found no evidence of specific co-localization of QTLs influencing male signal and female receiver traits on the same linkage groups. This finding suggests that the sexual selection process would proceed at a modest rate in A. grisella and that evolution toward exaggerated character states may be tempered. We suggest that this equilibrium state may be more the norm than the exception among animal species.

Keywords:*sexual selection, QTL, linkage map, sensory bias*

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Acoustic communication and dynamics of mating investments in bushcrickets (Orthoptera: Tettigoniidae)

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Tettigoniid males signal their willingness to mate through their mating songs, which give females the opportunity to choose between different males without direct contact. The songs are assumed to communicate the male's quality and fitness. One of the males' qualities is the transfer of large nuptial gifts at mating. A male's fitness might be reflected in his investment in song production and spermatophore investment. The perceived assessment of mating opportunities on the one hand and the potential risk of competition by rival males on the other hand make male tettigoniid reproductive investments highly dynamic. I present data about the adjustment in males' song production and spermatophore investment patterns with regard to a range of environmental factors such as population density, male-male competition and a male's condition.

Keywords:*Nuptial gifts, Katydids*

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S508W03

Genetics, Genomics and Evolutionary Entomology

Behavioral plasticity and host specialization in an acoustically orienting parasitoid

David Gray
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Ormia ochracea (Diptera: Tachinidae) is a specialized acoustic parasitoid of Gryllus field crickets. Gravid female flies locate calling male crickets, and larviposit on or near the host. Here I review recent work on cricket acoustics and the potential trade-off between host specialization and evolutionary lability. This work includes molecular genetics, field playback experiments in single-host and multiple-host populations, as well as laboratory playbacks manipulating prior acoustic experience of the flies. I also briefly consider the impacts of specialized acoustic predators on the evolution of acoustic communication in crickets.

Keywords:*Gryllus field crickets, Ormia parasitoid*

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Age and the effect of social interaction on animal personality

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Individuals in natural environment adjust their behaviour according to demographic factors. However, the longitudinal effects of social interactions on individual behaviour and behavioural syndromes have rarely been tested in animals. Here, male water striders (Hemiptera: Gerridae) were exposed to two different sets of conditions: the presence of only a single female (non-social treatment) or the presence of ten females and nine other males (social treatment). I then measured the consistency and plasticity of the following behaviours every two weeks over the lifetime of each male: dispersal ability, exploration in a novel environment, sex sensitivity (how quickly he recognized that another male is not a potential mate), and how long he persisted in attempting to mate a resistant female. All male personality traits except sex sensitivity varied consistently among males. Male dispersal tendency and exploratory behaviour remained more consistent over time in the social treatment than in the non-social treatment. Persistent mating attempts to resistant females were more consistent within the non-social treatment. In spite of behavioural consistency, males also varied in the plasticity of all behavioural traits. In particular, males from the social treatment become more likely to explore as they grew older, but the opposite was true of males from the non-social treatment. I conclude that the interaction between individuals contributes to the change of behavioural consistency and plasticity, potentially over the term of a male's life. Thus we suggest that demographic factors have an impact on the ecology and evolution of populations.

Keywords:*Phenotypic plasticity, personality, social interaction, age, water strider*

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Reproductive isolation between two closely related cricket species, *Gryllus fultoni* and *G. vernalis*

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Reproductive isolation between closely related taxa can occur via interruption of any stage of the reproductive process, prezygotic or postzygotic. However, a combination of several barriers is often required to eliminate costly reproductive interactions between closely related taxa. In eastern North America, two cricket species, Gryllus vernalis and G. fultoni (Orthoptera: Gryllidae), occur together in an area from eastern Kansas to northwest of the Appalachian Mountains. No hybrid offspring between two species were produced under laboratory conditions. Analyses of field and laboratory recordings showed that differences in pulse rate and chirp rate between the calling songs of the two species was greater in areas of sympatry than in areas of allopatry, a pattern expected of reproductive character displacement (RCD). The pattern of female mate discrimination in G. fultoni is also similar to the divergent pattern of calling songs between sympatric and far allopatric populations. At a common temperature, this female selectivity would be expected to significantly reduce heterospecific mating attempts in sympatry, consistent with predictions of RCD. Unlike those of G. fultoni, no comparable pattern of RCD is found in G. vernalis, resulting in asymmetry in RCD in this species pair. The final layer of premating reproductive isolation mechanism may be the discrimination by G. vernalis females against heterospecific males in close-range mating behaviors. Females of G. vernalis are selective with regard to mating, compared to those of G. fultoni. Thus, reproductive isolation occurs primarily in close-range mating behaviors in G. vernalis but in long-range mating behaviors in G. fultoni.

Keywords:*calling song, female mate discrimination, reproductive character displacement, reproductive isolation*

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S508W06

Genetics, Genomics and Evolutionary Entomology

Understanding reproductive isolation in duetting green lacewings through the quantitative genetics of song and preference

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Hybridization experiments can be used to estimate the genetic architectures underlying traits of interest as well as understand character homology of complex traits. Two cryptic species of green lacewings Chrysoperla agilis and C. carnea were crossed to identify inheritance patterns of species-specific mating signals and preferences for these signals. Multiple homologous genetic loci are hypothesized to underly both song features and preferences considering the recent origin of these species and the fact that these mating signals are the major reproductive isolation trait between the species. The inheritance of principal components describing hybrid song features fit additive (PC1), and additive plus dominance (PC2 & 3) models in joint scaling tests. Hybrids did not prefer the songs that most resembled the ones they themselves produced, and the variance in hybrid preference was greater than the variance in hybrid song features.

Keywords:*Hybridization experiments*

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Plasticity of predator avoidance affect optimal timing of aposematic signaling in prey: Predictions from agent-based modelling

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Warning sound to predators, or acoustic aposematism, can be evolved in several different ways in insects. Honeybees constantly emit buzzing sound, while certain moth caterpillars only click after an event of disturbance, and tiger moths utter ultrasonic warning at the final stage of bats' approaching behavior. An agent-based modeling technique was used to assess evolutionary prerequisites enabling each signaling strategy. The theoretical model newly predicted that if predator avoidance was learned rather than evolved, then preys tend to evolve a conditional or threat-based signaling strategy instead of more simplistic strategies. In addition, the model provided deeper understanding of evolutionary dynamics related to more conventional variables, including cost of signaling, predator learning speed and level of crypsis. Especially, level of crypsis was the strongest factor ruling signal conditions, unlike simple cost of signaling which mostly affected general strength of signaling only. These findings suggested that preys' investment to keep 'education' of predator population should be taken into consideration in predator-prey interaction studies, a step which has been widely neglected in previous studies.

Keywords:*Aposematism, Signalling, Predator-prey Interaction, Evolution, Agent-based Modelling*

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S508W08

Genetics, Genomics and Evolutionary Entomology

Divergence in matingdisplays predicted by divergence in mate preferences, not their strength

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After decades of intense research, the role of sexual selection in speciation remains unclear. We suggest that this ambiguity may be due to comparative tests of the effect of sexual selection on speciation not making a distinction between(i) the strength and(ii) the amount of divergence in the forces that causesexual selection. We address this distinction with taxonomically and methodologically diverse case studies of sexual selection via mate choice, including insects, spiders, frogs and fish. We relate quantitative descriptions of mate preferences to the distribution of sexual ornament traits, and find that ornament divergence is explained by the amount of divergence in mate preferences, rather than by their strength.This finding is intimately connected to speciation, because divergence in ornaments and mate preferences often directly influences assortative mating and reproductive isolation. We conclude that the effect of sexual selection on speciation may have been considerably underestimated to date.

Keywords:*mate preferences, matingdisplays*

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S509W01

Genetics, Genomics and Evolutionary Entomology

The *Musca domestica* genome and transcriptome analysis in relation to insecticide resistance

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House flies are cosmopolitan, ubiquitous, and potentially transmit many human and animal diseases. The Musca genome will enable us to study the macro- and microevolutionary process of adaptation of the house fly to the changing agricultural and urban environment. The house fly has a well-described linkage map and genome size is < 2-fold larger than D. melanogaster. The house fly genome will expand our understanding of house fly biology and help in developing new control strategies and new insect control agents based on new targets or manipulation of the unusual autosome-based sex determination system. Insect immunity, sex-determination, insecticide resistance as well as comparative genomics of the host-parasitoid system Musca-Nasonia will strongly benefit from the housefly genome sequence. The house fly is one of the insect species that has shown a great ability to develop resistance to insecticides and has been followed in our lab from organchlorines to spinosad. Comparison of transcriptome (400.000 sequences averaging 250 bp) from insecticide-resistant and susceptible house fly strains revealed interesting difference in cytochrome P450 genes as well as other stress and toxicology related genes. The emergence of >30 insect genomes and an overwhelming amount of transcriptome data, now including the house fly genome and transcriptome data, during less than a decade will change insect research, as large amounts of data will rapidly become available. Studies of Musca ecology, physiology, pest control etc. will enter the genomic era. The benefits and the options of having the house fly genome at hand will be discussed.

Keywords:*Musca, genome, transcriptome, insecticide resistance*

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Sex and the housefly: Evidence for a conserved bifurcation of the sex-determining pathway

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The genetic pathway, which specifies neuronal functions and circuitries required for proper male courtship performance, has been extensively studied in Drosophila. In this insect model, the gene fruitless (fru), a direct target of the sex-determining cascade, has been identified as a key regulator of dimorphic development of the CNS. Little is known about the genetic control of sex-specific behaviours in other insects. We will present evidence that the fru ortholog in the housefly, Md-fru, is also required for proper male courting behaviour. Md-fru is a direct target of the sex-determining cascade in the housefly. Md-fru expresses functional products in male individuals in which the sex-determining switch gene transformer (Md-tra) is functionally OFF. Individuals, in which Md-tra is functionally ON, are instructed to develop into females and display no courting behaviour. Here, Md-tra causes a change in Md-fru splicing which leads to the incorporation of in-frame stop signals that render Md-fru functionally OFF. We characterized a mutant strain in which residual activity of a hypomorphic allele of Mdtra causes severely impaired courtship performance in homozygous males. While Md-dsx, the prime effector of the sex determining pathway is not affected by residual activity of Md-tra and is expressed in its proper male mode, Md-fru is spliced in the non-functional female mode. Removal of the residual Md-tra activity by RNAi shifts Md-fru back into its functional male mode of expression and reconstitutes normal male

Keywords:*Musca domestica, sex determination, mating behavior, fruitless*

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Genetics, Genomics and Evolutionary Entomology

The quest for the elusive male determining factors in the housefly

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The housefly is an exceptional organism in having populations with different sex determination mechanisms, including the standard male heterogametic (XX-XY) system, location of the dominant male determiner (M) on different autosomes, or female heterogamety. The frequency of autosomally located M factors shows worldwide clinal variation, being more abundant at higher temperatures. We will present approaches to identify the male determining gene(s) and to test theories about recombination suppression in neo-sex chromosomes. We will cross autosomal M strains to mutant laboratory strains to map, isolate and characterise M factors. We will exploit the Housefly Genome Project to develop dense marker linkage maps and identify candidate genes for functional analyses. We will measure local and genome wide recombination frequencies in strains with different sex determination mechanisms and establish temperature effects on sex determination gene expression (transcriptomics). We aim to gain a better understanding of the mechanisms and evolution of sex determination mechanisms in the housefly.

Keywords:*housefly, M factor, sex determination*

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Age-dependent manifestation of sex-linked wings fragility mutation in *Musca domestica*

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Identification of age-dependent changes of morphological deviations caused by mutations is an important experimental basis for investigations of genetic determination of aging and longevity. I present the description of new mutation causes the fragility of wings in house fly Musca domestica L. adults. Mutation "fragile wings" (fw) manifests in males only since 14-21 days after adults eclosion. Crosses between males with fragile wings and virgin females from original strain Cooper during 7 generations allowed to reveal the absence of fw phenotype in females. Female/male ratio in progenies is about 1:2. We proposed that mutation linked with X-chromosome and appears to be lethal for females when is homozygous. In laboratory house fly strains differing by life span (average life span of adults is 32-37 days in the strains Sh 28 and Sh gen, 47-52 days in strains L 2 and I gen) the significant share of males with fw phenotype was founded (0.25-0.55). This phenotype is expressed as the trait limited by sex. Further investigations required for elucidating the genetic basis of this trait. Investigations funded by Russian Foundation for Basic Research (grants 11-04-97005-r_povolzhye_a, 10-04-01614-a).

Keywords:*Musca domestica, mutation, age-dependent manifestation, fragile wings*

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The transcriptome of hytrosavirus infected *Musca domestica*

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Initially, the Roche's 454 GS-FLX system provided sequence on the Musca domestica hytrosavirus, a novel dsDNA virus that infect and sterilize various dipteran pest insects. Putative ORFs identified in the 125kbp MdSGHV were confirmed using both 3' RACE and qPCR. A second series of cDNA preparations were subjected to adaptor ligation, clonal hybridization to DNA capture beads and emPCR. Sequencing with the GS FLX titanium chemistry produced a total of 250,000 clean reads out of ~1x106 wells. The 37,449 assemblies were examined for contaminations (rRNA, mitochondrial reads) and the remainder annotated with NR blast hit, NT blast hit, Gene Title and GeneOntology terms. Analysis of the sequence data generated from the non-normalized library of cDNA revealed the presence of both housefly and MdSGHV transcripts. Quantitative analysis of the viral reads demonstrated the presence of abundant (18), moderate (29), and rare transcript classes (51). Interestingly, eight of the putative ORFs identified in silico were not detected in the sequences generated. After further filtering, a total of 37,449 assembled sequences were identified in the database of which 31,535 assemblies mapped to Drosophila homologues. Further annotation (housefly 454ASSEM_NR) revealed that of the 37,449 total, 14,590 queries had hits with e-values ≤ 0.0001; 9,352 with e-values between 0.0001 and 10, and 13,507 had no hits (e-values >10). The number of ESTs generated, although not as great as that generated from a normalized library, provided quantitative data on viral transcript abundance and a wealth of sequence data on house fly genes.

Keywords:*Hytrosavirus,*

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Opportunities and challenges of non-model ecoinformatics

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Newer generation sequencing of co-occurring species with known ecology has transformative research potential and offers great opportunities for novel bioinformatics. Examples of such research include studying recent speciation, adaptation to climate change, and large-scale metagenomics in ecological settings. Here, we focus on analysis of co-occurring natural populations starting with our on-going and published transcriptome experience with two wild butterflies: the Propertius duskywing, \emph{Erynnis propertius} (Lepidoptera: Hesperiidae) and the Anise swallowtail, \emph{Papilio zelicaon} (Lepidoptera: Papilionidae). We compare and contrast these data with related restriction enzyme-associated sequencing (i.e., RADtaging) applied to genetic mapping and genome analysis of similarly sampled fruit flies (\emph{Rhagoletis} genus). We then describe opportunities and challenges of these datasets derived from two distinct newer generation sequencing platforms and their use in ecological informatics. This work is tied to our NIH/NIAID bioinformatics resource VectorBase that also is developing population genetics tools for vectors of human disease, All analysis is performed using our general and scalable framework that includes, among other tools, the discovery of SNPs, natural population haplotyping, and large-scale annotation of diverse environmental samples. We show runtime results of this framework using Amazon EC2, SGE, Condor and mixtures of the three. Finally, we share biological insights aided by our newer generation ecoinformatics including adaptation in malaria mosquitoes, differential responses to climate change of wild butterflies, and preliminary population genetics of field-caught Rhagoletis fruit flies. These applications involve model and non-model organisms and differences in the analysis will be discussed.

Keywords:*next-gen sequencing, RADtag. bioinformatics*

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Analyzing biodiversity for bulk insect samples using Illumina NGS

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Classic DNA barcoding protocols have been optimized for individual processing of biological samples. Next Generation Sequencing (NGS) technologies are considered as a potential solution to overcoming hurdles in data collection with accurate analysis of environmental samples of mixed taxa. Among current NGS platforms, Illumina sequencers have the potential to provide an efficient solution to analyzing environmental samples at much lower cost per sequence read. In this study, we first sequenced mixed amplicons for the standard barcode region obtained from insect samples collected by Malaise traps. Additionally, we developed a PCR?independent protocol to overcome taxonomic bias introduced by PCR amplifications.Our results show that the improved sequence length and the large sequencing capacity currently available on Illumina NGS has ensured its application to environmental biodiversity analysis, with several technical advantages: higher sensitivity for taxa with low abundance, higher recovery ratio for true positives, lower running cost per sample, and the capability to eliminate PCR bias. The application of Illumina platforms in bioassessment is promising, yet modifications of protocols in collection, preservation, and molecular lab work seem critical for this implementation.

Keywords:*environmental barcoding, biodiversity, biomonitoring, NGS*

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S510TH03

Genetics, Genomics and Evolutionary Entomology

Sex, Y genes and gene duplications in *Anopheles mosquitoes*: Integrating bioinformatics, next-gen sequencing, and digital droplet PCR

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The application of the so-called 2nd-generation sequencing technologies is having a profound impact on mosquito genetic research. Here I will first briefly discuss our efforts to obtain a draft Anopheles stephensi genome assembly by combining the 2nd-generation sequencing and a novel Focused Genome Sequencing approaches. I will then focus on a new strategy to integrate next-gen technology and bioinformatics to identify and characterize Y-chromosome genes and gene duplication events in Anopheles species.

Keywords:*genomics, 454, illumina, Y genes, bioinformatics, ddPCR*

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Strain variation in the transcriptome of the dengue fever vector, *Aedes aegypti*

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Studies of whole-genome transcript modulation provide a basis for understanding functional elements of the genome and the complexity of gene regulation. Aedes aegypti, a significant vector of both dengue and yellow fever viruses, is adaptable to diverse ecological conditions and exhibits variation in vectorial capacity to arboviruses. Population genetic studies record a varying degree of world-wide genetic polymorphism, however, the extent of functional genetic polymorphism across strains was previously unknown. Transcriptomes of three Ae. aegypti strains, Chetumal (CTM), Rexville D-Puerto Rico (Rex-D) and Liverpool (LVP) were compared. CTM is more susceptible than Rex- D to infection by dengue virus serotype 2. Transcripts enriched in blood-fed mosquitoes encode proteins associated with catalytic activities, molecular transport, metabolism of lipids, carbohydrates and amino acids, and functions related to blood digestion and the progression of the gonotropic cycle. Significant qualitative and quantitative differences were found in individual transcripts among strains including differential representation of paralogous gene products. The majority of immunity-associated transcripts decreased in accumulation after a bloodmeal and the results are discussed in relation to the different susceptibility of CTM and Rex-D mosquitoes to DENV2 infection.

Keywords:*Aedes aegypti, strain variation, bloodmeal, RNA-seq*

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De-novo transcriptome sequencing to identify the molecular underpinnings of seasonal adaptation in the Asian tiger mosquito, *Aedes albopictus*

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Many temperate insects survive the harsh conditions of winter by undergoing photoperiodic diapause, a pre-programmed developmental arrest initiated by short day lengths. To discover transcripts involved in diapause preparation, we sequenced and assembled the transcriptomes of Aedes albopictus early- (3 d post-oviposition) and late- (6 d post-oviposition) stage embryos reared under diapause-inducing or non-diapause-inducing conditions. Illumina Hi-Seq yielded ~120 million paired-end reads, which we combined with a previous assembly of 454-GS FLX reads derived from Ae. albopictus oocytes. Because an annotated genome sequence for Ae. albopictus is not currently available, we took advantage of strong sequence similarity to the genome sequence of Aedes aegypti, as well as other reference organisms, to annotate our assembly. We identified 12,293 ESTs with high-confidence annotations to known Dipteran gene models. Analyses of differential expression revealed strong ontogenetic changes in gene expression between early- and late- stage embryos in both diapause-destined and non-diapause destined embryos. While not as prevalent, gene expression differences between diapause-destined and non-diapause-destined embryos were present in both developmental stages, and were more pronounced later in development. Genes and pathways related to morphogenesis, metabolism and stress response were among the most strongly differentially expressed between diapause- and non-diapause-destined embryos in both developmental stages. Our analysis illustrates a useful approach that draws from closely related reference genomes to generate high-confidence ESTs in a non-model organism.

Keywords:*diapause, Aedes albopictus, next-generation sequencing*

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Genomic dissection of *Wolbachia*-dengue interactions in mosquitoes

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Diseases transmitted by blood-feeding arthropod vectors, such as mosquito-borne malaria and dengue fever, cause 1.5 million human deaths every year. The insufficiency of currently available strategies, including vaccines, drugs, and pesticides, has led to an increase in vector-borne diseases. The endosymbiotic bacterium Wolbachia is widely recognized for its potential as a vehicle to introduce disease-resistance genes into mosquitoes, making them refractory to the human pathogens they transmit. This is due to its ability to induce both a reproductive abnormality known as cytoplasmic incompatibility and a resistance to human pathogens including dengue virus (DENV) in mosquito. Here, we utilized several genomic tools to dissect molecular mechanism of this Wolbachia-mediated resistance to DENV. The microarray assay indicates that transcripts of genes with functions related to immunity and Redox are up-regulated in Ae. aegypti infected with Wolbachia. Infection with this bacterium leads to induction of oxidative stress and an increased level of reactive oxygen species (ROS) in its mosquito host. ROS elevation is linked to the activation of the Toll pathway, which is essential in mediating the expression of antioxidants to counterbalance the oxidative stress. This immune pathway is also responsible for activation of antimicrobial peptides (AMP), defensins and cecropins, which are involved in inhibition of DENV proliferation in Wolbachia-infected mosquitoes. Finally, three-way interactions between Wolbachia, DENV and mosquito will be analyzed in mosquito protein interaction network to further understand the mechanisms of viral interference. We will discuss the significance of our findings for development of novel strategies for control of vector-borne diseases.

Keywords:*Wolbachia, dengue, mosquitoes, symbiosis*

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Genomic analysis of inversion breakpoints revised the chromosomal phylogeny and evolutionary history of African malaria mosquitoes

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Malaria mosquitoes often belong to complexes of morphologically indistinguishable sibling species that differ in geographic distribution, ecological adaptation, host-seeking behavior, and the ability to transmit a parasite. Understanding phylogenetic relationships within complexes is crucial for identifying the genomic changes associated with the evolution of epidemiologically important traits. To determine ancestral chromosomal arrangements in the An. gambiae complex, we identified breakpoints of fixed overlapping chromosomal inversions 2Rop of An. merus using fluorescence in situ hybridization, a whole-genome mate-paired sequencing, and clone sequencing. We also mapped breakpoints of a chromosomal inversion 2La in outgroup species using a bioinformatics approach. We demonstrated that the "standard" 2R+p arrangement and "inverted" 2Ro and 2La arrangements are present in three outgroup species, Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. The data indicate that the ancestral species of the An. gambiae complex had the 2Ro+p and 2La chromosomal arrangements. The "inverted" 2Ro arrangement uniquely characterizes a malaria vector An. merus as the basal species in the complex. The rooted chromosomal phylogeny implies that the ancestral species gave rise to An. merus by generating the 2Rp inversion and to its sister species An. gambiae by generating the 2R+o inversion. We conclude that vectorial capacity had evolved repeatedly in the complex because it was lost in the intermediate An. quadriannulatus lineage and was originated again in the terminal An. arabiensis and An. melas lineages.

Keywords:*Anopheles gambiae complex, mate-paired sequencing, chromosomal inversions, breakpoints, malaria mosquitoes*

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S510TH08

Genetics, Genomics and Evolutionary Entomology

Comparative genomics of insect pathogenic fungi

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There are about one thousand of known fungal species capable of killing insects. The species like Metarhizium anisopliae, M. robertsii, M. acridum and Beauveria bassiana has been developed as environmentally friendly biological control agents against different insect pests. The species of the caterpillar pupae specific fungus Cordyceps militaris, however, has been used a traditional Chinese Medicine for hundreds of years. We sequenced the genome of Metarhizium spp., B. bassiana and C. militaris to better understand the evolution of fungal entomopathogenicity and provide genetic resources to facilitate its exploitation. The insect pathogens have a strikingly larger proportion of genes encoding secreted proteins, particularly proteases, than other sequenced fungi. A phylogenomic analysis confirmed that entomopathogenicity is polyphyletic so similar expansion of families of proteases and chitinases reflects a convergent evolution. Nonetheless, the presence and absence of key virulence-related genes and the expansion and contraction of protein families in different insect pathogens suggest that fungi evolved diverse strategies to kill insects and or maintain host range. Like C. militaris, the sexualities of Metarhizium and Beauveria species are verified to be heterothallic. High throughput transcriptome analysis indicated that the pathogens could regulate transcriptional responses with fine-tuned gene sets for host recognition and adaptation to different niches. The information from our studies advanced the understanding of the evolution of fungal entomopathogenicity and will benefit future molecular studies of fungus-host interactions and thereby facilitate the development of vector pathogens as cost-effective environmentally friendly mycoinsecticides.

Keywords:*Insect pathogenic fungi, comparative genomics, insect-fungus interactions*

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S510TH09

Genetics, Genomics and Evolutionary Entomology

Identifying microRNA genes from five important insect pests

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Small RNAs are an important class of non-coding RNAs, including miRNA (microRNA), piRNA (piwi associated RNA), siRNA (small interfering RNA) and long non-coding RNA. In past decades, messenger RNA (mRNA) played major roles in molecular biology research because they encode proteins. Discovery of microRNA and piRNA opened a new era of noncoding RNA study . MicroRNA is one of the most important class of noncoding RNA genes, which involved in the growth of organisms, cell differentiation and apoptosis, tumor formation and occurrence of cancer and other life processes. Unfortunately, insect microRNAs has been less understood. In insect miRNA research filed, finding small RNA genes is still the important task. The traditional experimental methods is time-consuming and laborious. The deep sequencing technology provides a powerful tool to identify small RNA genes at a large-scale level. In this work, we sequenced small RNAs of five important agricultural pests, Tryporyza incertulas (walker), Spodoptera exigua Hiibner, Plutella xylostella(L.), Nilaparvata lugens(Stal), Laodelphgax striatellus. A comprehensive pipeline was developed to discover miRNAs by comparative genomics and machine learning methods. The genome of Bombyx mori and Acyrthosiphon pisum were used as the reference. As a result, 188 microRNAs were found, which has homoloies in the miRBase database. Fifty two microRNA were successfully identified by the software mireap using the reference genome. Combining transcriptome and small RNA library data, 41microRNA were successfully detected using miRDeep.

Keywords:*Small RNAs*

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S510TH10

Genetics, Genomics and Evolutionary Entomology

Functional genomic analysis of chelicerate species

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Arthropoda comprises two major branches, Chelicerata and Mandibulata, which arose from Cambrian period and radiated into millions ofspecies over 400 millions of years. A comparative study was performed on transcriptome data from two arachnids, eleven insects, a crustacean, and two chordates. A phylogeny tree was built based on maximum likelihood analyses of orthologs in the eukaryotic core gene set. Chelicerates shared 3,806 gene families, and had 8,964 unique gene families. On the other hand, insects shared 676 gene families, and had 17,700 gene families specific to their own. Extending the analysis to include chelicerates, hexapods and crustacean, their common and unique genes highlight the genetic basis for many important biological features. The genetics repertoire of arthropods is likely the balanced outcome of gene accumulation and decay through a long history of evolution. This study offered insights into the evolution of chelicerates and arthropods.

Keywords:*Chelicerata and Mandibulata*

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Genome scale evolutionary analysis for revealing the oldest bacterial organism on the Earth

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We constructed a phylogenetic tree of 17 bacterial phyla covering eubacteria and archaea by using a new method and 102 carefully selected orthologs from their genomes. One of the serious disturbing factors in phylogeny construction is the existence of out-paralogs that cannot easily be found out and discarded. In our method, out-paralogs are detected and removed by constructing a phylogenetic tree of the genes in question and examining the clustered genes in the tree. We also developed a method for comparing two tree topologies or shapes, ComTree. Applying ComTree to the constructed tree we computed the relative number of orthologs that support a node of the tree. This number is called the Positive Ortholog Ratio (POR). Our result of bacterial phylogeny analysis is consistent with previous ones showing that hyperthermophilic bacteria such as Thermotogae and Aquificae diverged earlier than the others in the eubacterial phylogeny studied. It is noted that our results are consistent whether thermophilic archaea or mesophilic archaea is employed for determining the root of the tree. The earliest divergence of hyperthermophilic eubacteria is supported by genes involved in fundamental metabolic processes such as glycolysis, nucleotide and amino acid syntheses.

Keywords:*Genomic, bacterial organism, Bioinformatics*

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S511TH01

Genetics, Genomics and Evolutionary Entomology

Bugs and drugs and speciation: The impact of mutualistic *Wolbachia* on *Drosophila* development, sexual behavior and symbiotic speciation

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In speciation research it is still an ongoing debate over whether or not, and if yes to what extend, ubiquitous microbial reproductive tract symbionts have the ability of manipulating host sexual behavior in nature and - in their long-term consequence - of fostering biodiversity. We recently have reevaluated the role of native endosymbiotic bacteria Wolbachia on directing assortative mating in females as well as on pheromone expression and perception in both sexes in the neotropical Drosophila paulistorum species complex trapped mid-evolution in flagrant statu nascendi. Here we show that obligate mutualistic Wolbachia foster incipient host speciation by triggering both pre- and postmating isolation. As broadly accepted the actual formation of a new species is a slow process, generally requiring many generations, and therefore one cannot expect to artificially create vicariant speciation in the laboratory to yield substantial reproductive isolation. However, the D. paulistorum species complex presents an exceptional case providing compelling experimental evidence for any spontaneous creation of a new Drosophila species in the laboratory. As reported by DOBZHANSKY the D. paulistorum strain Llanos was originally compatible in crossings with Orinocan semispecies, but after five years in the laboratory this strain spontaneously had transformed into a new semispecies with complete hybrid male sterility. We have established artificial Wolbachia 'depletion-restoration' lines of D. paulistorum via antibiotics. Such lines consistently express strong de novo mate avoidance against parental untreated partners. Molecular and cellular mechanisms will be discussed triggering alterations in sexual behavior in response to artificial disturbances of the native host-symbiont equilibrium.

Keywords:*speciation, mutualist, pathogen, sexual behavior, pheromone*

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S511TH02

Genetics, Genomics and Evolutionary Entomology

Symbiont-mediated body colour change in aphids

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In the pea aphid Acyrthosiphon pisum, red and green color morphs are found in the same populations. Early ecological studies showed that the color variation has some important traits, often involved in the prey-predation relationships. Recently we found an unexpected layer underlying aphid color polymorphism: novel aphid endosymbiont of the genus Rickettsiella is responsible for green body color in at least some green pea aphids in natural populations. Diagnostic PCR surveys in Western Europe, North America, and Japan revealed that prevalence of Rickettsiella was significantly different between those regions. Infection experiments of various Rickettsiella strains indicated that phenotypic effects of Rickettsiella infection on the body color were slightly different but they similarly changed the red body color into green. Aphid body color mainly consists of yellow-red carotenoid and blue-green polycyclic quinones. We could not find any significant changes of carotenoid compositions by HPLC and gene expression analyses. Meanwhile, TLC analysis suggested that Rickettsiella infection increased amounts of green polycyclic quinones and no new pigments appeared upon the Rickettsiella infection. We chemically characterized the main green pigments, and developed highly specific and sensitive detection method of the green pigments by using LC-MS. LC-MS analyses confirmed that both of Rickettsiella-infected and uninfected aphids contain same green pigments, although the amounts of the pigments were remarkably different between them. From those results, Rickettsiella probably does not synthesize the green pigments by itself but somehow interferes with the aphid metabolism to activate the green pigment production.

Keywords:*body color, polymorphism, pea aphid, Acyrthosiphon pisum, Rickettsiella*

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Invited submission (session 5-6 Advances in symbiosis, talk S6-4): Evolution of symbiont-mediated antibiotic defense in solitary wasps

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Mutualistic microorganisms play a key role for nutrition or pathogen defense in many insects. Beewolves (digger wasps of the genera Philanthus, Trachypus and Philanthinus) engage in a unique symbiosis with Streptomyces bacteria that they cultivate in specialized glands in their antennae and transfer them to the larval cocoon. On the cocoon, the symbionts provide protection for the larva against fungal and bacterial pathogens by producing a complex cocktail of antibiotic substances. Thus, by means of their symbionts, beewolves employ a strategy comparable to the combination treatment used in human medicine as an efficient defense for their offspring against a broad range of detrimental microorganisms. We reconstructed multi-gene phylogenies for both symbiotic partners to elucidate the evolutionary history of this remarkable association. Molecular clock calibrations based on the fossil record of crabronid wasps indicate that the symbiosis evolved at least 65 million years ago. Thus, the association is more ancient than any other defensive insect-bacteria mutualism known to date. The beewolf symbionts form a monophyletic clade within the genus Streptomyces, suggesting a single infection event and subsequent co-diversification of hosts and symbionts. However, comparative phylogenetic analyses provide strong evidence for frequent horizontal transfer of symbionts, possibly by interspecific predation or nest re-use among beewolf species. Thus, defensive symbioses with extracellular bacteria can remain stable over long evolutionary timescales even in the absence of co-cladogenesis, demonstrating that strict vertical transmission is not a prerequisite for the maintenance of protective mutualisms.

Keywords:*Streptomyces, Defensive symbiosis, Hymenoptera, Antibiotics*

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Insecticide resistance governed by endosymbiontic bacteria

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Development of insecticide resistance has been a serious concern worldwide, where insecticide abuse results in evolutionary changes in the pest insect genomes such as alteration of drug target sites, up-regulation of degrading enzymes, enhancement of drug excretion, etc. Here we report a previously unknown mechanism of insecticide resistance: infection with insecticide-degrading bacterial symbiont immediately establishes insecticide resistance in pest insects. The bean bug Riptortus pedestris and allied stinkbugs are in mutualistic association with Burkholderia gut symbionts, which are acquired by nymphal insects from the soil environment. In agricultural fields, fenitrothion-degrading Burkolderia strains are present at very low densities. We demonstrated that the fenitrothion-degrading Burkholderia strains are able to establish a specific and beneficial symbiosis with the stinkbugs and confer a resistance of the host insects to fenitrothion. Experimental applications of fenitrothion to field soils drastically enriched fenitrothion-degrading bacteria from 0% to 80-90% of total culturable bacterial counts, and over 90% of stinkbugs reared with the enriched soil established symbiosis with the fenitrothion-degrading Burkholderia. In a Japanese island where fenitrothion has been regularly and constantly applied to sugarcane fields, we identified a stinkbug population wherein the insects live on sugarcane and around 8% of them host fenitrothion-degrading Burkholderia. Our finding may entail practical relevance in that the symbiont-mediated insecticide resistance can develop even in the absence of pest insects and quickly establish within a single insect generation.

Keywords:*Insecticide resistance, Symbiotic bacteria, Mutualism, Burkholderia, Heteroptera*

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S511TH05

Genetics, Genomics and Evolutionary Entomology

The role of the innate immune system of the ant *Camponotus floridanus* in regulation and maintenance of its endosymbiont *Blochmannia*

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Numerous insect species harbour mutualistic endosymbiontic bacteria. Insect hosts face the problem of having to maintain such mutualistic bacteria while staging an immune response towards pathogens upon infection. Camponotus floridanus ants harbour the obligate endosymbiont Blochmannia floridanus in specialized midgut cells and ovaries. During the holometabolous life cycle of the ant hosts the distribution of bacteriocytes and of Blochmannia endosymbionts is remarkably dynamic. The proportion of cells of the midgut harbouring Blochmannia increases strongly during host ontogeny and peaks in late pupal stages, where the entire midgut is transformed into a symbiotic organ. After eclosion of workers the symbiosis degenerates as bacterial numbers decreases with age of workers. We identified genes transcriptionally induced in response to septic injury by suppression subtractive hybridization. Among these were genes involved in pathogen recognition, signal transduction, antimicrobial activity, or general stress response. A quantitative analysis of immune gene expression revealed different expression kinetics of individual factors and characteristic expression profiles after injection of gram-negative and gram-positive bacteria. B. floridanus injected into the hemocoel elicited a comparable immune response of its host C. floridanus. Furthermore, in untreated hosts some immune genes displayed distinct expression patterns according to developmental stage and tissue. Thus, the host´s immune system may prevent colonization of tissues other than the bacteriocytes by Blochmannia and may also play a role in the regulation of the number of bacteria present within bacteriocytes

Keywords:*endosymbiont*

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Symbiont-mediated invasive success of the Harlequin ladybird *Harmonia axyridis*

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An increasing number of invasive species characterized by rapid growth and spread of populations after their establishment in new habitats impact negatively both biodiversity and economy. Their invasive success is promoted by several factors among which a strong immune system has been postulated because invaders have to cope with pathogens which they face in their new environments. We selected the harlequin ladybird Harmonia axyridis to explore the role of immunity in invasion biology. Its remarkable capacity to outcompete native ladybird species upon introduction into new habitats has been linked to its prominent resistance against pathogens. We discovered a strong constitutive antibacterial activity in the hemolymph of H. axyridis beetles which has been attributed to the chemical defense compound harmonine exhibiting a broad-spectrum activity against pathogens including those causing human diseases such as the infective agents of tuberculosis and malaria (Rohrich et al. 2012, Biology Letters). Surprisingly, injection of antibiotics decreased the antimicrobial activity in the hemolymph implicating the participation of symbionts in chemical defense of H. axyridis.

Keywords:*Harmonia axyridis, invasion biology, immunity, chemical defense,*

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Prevalence of the symbiont *Cardinium* in *Culicoides* (Diptera: Certopogonidae) vector species.

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Infection prevalence of symbionts in arthropod hosts may vary according to several reasons among are the environmental conditions affecting the host. Here we identified and localized the symbiont 'Candidatus Cardinium hertigii' in two major livestock disease vector species of Culicoides biting midges (Diptera: Ceratopogonidae) from Israel, determined its geographic prevalence and its association with climatic factors. The prevalence of adult infection differed significantly between C. imicola and C. oxystoma, though phylogenetic analyses showed that Cardinium in these species are almost identical. The prevalence of infection differed significantly between climate regions with lowest prevalence in the arid region and highest prevalence in the Mediterranean region. In addition, multivariate linear regression analysis of Cardinium prevalence together with climatic and satellite imagery data-derived environmental variables revealed that infection prevalence is significantly and strongly associated with land surface temperature. These findings suggest that the observed variation of Cardinium infection of the imago stage of Culicoides species may be influenced by environmental conditions affecting the latter's early developmental stages.

Keywords:*biting midge, insect symbiosis, satellite imagery data, vector ecology*

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S511TH08

Genetics, Genomics and Evolutionary Entomology

Repeated interactions and the evolution of dependence in insect symbiosis

Fabrice Vavre
Biometry and Evolutionary Biology Lab, France

Numerous insects interact with vertically transmitted symbionts that exhibit very diverse effects. Many of these symbionts are mutualists and bring important advantages to their hosts, notably through the complementation of their diet. Others manipulate the reproduction of their host to their own advantage. Such manipulations include feminization of genetic males, male-killing, induction of parthenogenesis or cytoplasmic incompatibility. These two types of symbiosis have classically been studied separately, but recent advances show that this dichotomy is not as pertinent as previously thought. First, it is becoming clear that reproductive manipulators can also provide advantages to their hosts. Second, some examples show that the dependence of the host upon its symbiont may rapidly evolve even when the symbiont does not bring benefit to its host, either through conflict resolution or evolution of tolerance. Finally, evolution of dependence may also explain why infection by multiple symbionts within the same host may persist in the long term. These results provide interesting new scenarios for the evolution of obligatory host-symbiont relationships and the building of complex symbiotic communities. They suggest that dependence can emerge from different evolutionary routes and may stabilize host-symbionts interactions.

Keywords:*coevolution, tolerance, redundancy, Wolbachia*

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Tsetse-*Wolbachia* symbiotic associations

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Wolbachia is a widespread and highly diverse group of alphaproteobacteria infecting arthropod and filarial nematode species. These symbionts are frequently found as intracellular and maternally inherited symbionts in insect species, whose reproductive properties they manipulate by inducing cytoplasmic incompatibility (CI), parthenogenesis, male-killing and feminization. Due to their unique biology, Wolbachia symbiotic associations are currently being considered as promising tools for the control of agricultural pests and medical disease vectors, including Glossina sp., which transmit African trypanosomes, the causative agents of the sleeping sickness in humans and of nagana in animals. In the present study, we report on recent studies on the detection and characterization of Wolbachia infections in laboratory and natural populations of Glossina species, the horizontal transfer of Wolbachia genes to tsetse chromosomes and also on how Wolbachia symbiosis could be harnessed for the development of novel symbiont-based pest and disease control strategies.

Keywords:*Wolbachia, Glossina, insect symbiosis, African trypanosomosis*

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Symbionts and fitness of Tephritid fruit flies

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I will review recent work on the relationship between true fruit flies (Diptera: Tephritidae) and their associated microorganisms. Mediterranean fruit flies (Ceratitis capitata), harbor a diverse community of bacteria in their digestive system. Molecular and culture- based techniques show that members of the Enterobacteriaceae form the dominant populations in the medfly's gut. Among them, many are diazotrophs and actively fix nitrogen in vivo. Most prominent are Klebsiella spp, Enterobacter spp., Pectobacterium spp. Citrobacter freundii and Providencia stuartii. A marked shift in community composition was observed between different developmental stages: in larvae, the pectinolytic Pectobacterium were most abundant, suggesting that pectinolysis plays a role early during the fly's life. The presence of the intact bacterial community is significantly associated with egg laying in females, mating success in males and longevity in both sexes. The closely related olive fly (Bactrocera oleae), is intimately associated with one microorganism - Ca. Erwinia dacicola. This species contributes to adult nutrition by supplying essential amino acids, and allows larvae to overcome the phytochemical defenses of the olive. In conclusion, the association between bacteria and fruit flies is intricate and multi-faceted, yet evidence for interdependence between hosts and symbionts is very strong. Further research will unravel the specific manner whereby bacteria contribute to the fitness of their host, and how this association may be manipulated in control operations.

Keywords:*Symbiosis, Bacteria, Tephritidae, Co-Evolution*

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S511TH11

Genetics, Genomics and Evolutionary Entomology

Identification of a new secondary symbiont in the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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The whitefly Bemisia tabaci (Hemiptera: Aleyrodidae) is a species complex containing > 20 cryptic species some of which are important crop pests worldwide. Like many other sap-sucking insects, whiteflies harbour an obligatory symbiont Candidatus Portiera aleyrodidarum and a number of secondary symbionts. So far six types of secondary symbionts have been identified in B. tabaci including Hamiltonella, Arsenophonus, Wolbachia, Rickettsia, Cardinium and Fritschea spp. In this study, we found a novel bacterium in the indigenous B. tabaci cryptic species China 1 (previously known as B. tabaci biotype ZHJ3). Phylogenetic analysis based on the 16S rRNA gene showed that the bacterium belongs to the α-subdivision of the Proteobacteria and has a relationship with human pathogens Orientia spp. and secondary endosymbionts of the wheat aphids Sitobion miscanthi and Rhopalosiphum padi. As a result, we temporarily describe it as Orientia like organism (OLO). OLOs were found in 7 of 9 wild populations of B. tabaci cryptic species China 1 with an infection rate from 46.2% to 76.8%. Fluorescence in situ hybridizations (FISH) in nymphs and adults of B. tabaci China 1 revealed that OLOs are confined in the bacteriome and co-localize with Ca. Portiera aleyrodidarum. This study broadens the diversity of symbionts detected so far in B. tabaci.

Keywords:*Orientia, Bemisia tabaci, symbiont*

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Molecular and cellular mechanisms of self-sacrificing gall repair by soldier nymphs in the social aphid

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National Institute of Advanced Industrial Science and Technology (AIST), Japan

Together with bees, wasps, ants and termites, aphids contain many social species with individuals altruistic to their colony mates, called soldiers. In the social aphid Nipponaphis monzeni, gall repair is among the important tasks of soldiers for colony defense. N. monzeni forms completely closed galls on the tree Distylium racemosum. Although closed galls are generally resistant to predators, some gall-feeding specialists such as moth larvae often invade the gall by tunnelling through the wall. When a hole is bored on the gall wall, the soldier nymphs immediately gather around the hole, discharge a large amount of body fluid from their cornicles on the damaged area, and mix the fluid with their legs. The discharged fluid soon becomes viscous and solidifies, whereby the hole is filled up completely. In an attempt to understand the molecular basis of this behavior, especially the mechanism of the body fluid solidification, we analyzed protein components of the secretion. We found that the secretion contained six major components, one of which was phenoloxidase, a key enzyme involved in melanization and scab formation in insects. The phenoloxidase was expressed in peculiar, soldier-specific "large globular cells" that occupied the body cavity of the soldier nymphs. RNA sequencing analysis of the large globular cells revealed that many genes involved in the process of melanization were predominantly expressed in these cells. We suggest that aphid's innate immune and wound-healing mechanisms might have been enhanced and recruited to the social task, gall repair, in the lineage leading to N. monzeni.

Keywords:*social aphid, soldier nymph, gall repair, melanization*

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The genome of the socially-polymorphic halictid bee, *Lasioglossum albipes*

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Halictid bees, or "sweat" bees (family Halictidae), are an ideal system to study social behavior because they span the entire range of behaviors, from solitary to eusocial. Within this monophyletic family, there have been at least three independent origins of eusociality and many subsequent losses. As a result, the behavioral variation within this group encapsulates nearly all forms of social structure, including solitary, communal, and primitively eusocial species. Our work focuses on the halictid bee, Lasioglossum albipes. L. albipes exhibits variation in social structure across its range; some populations are primitively eusocial while others are solitary. Previous studies have demonstrated that this behavioral polymorphism is the result of a reversion from eusocial to solitary behavior, and that it has a strong genetic component. We are using population and evolutionary genomic approaches to identify some of the genetic and environmental variables associated with social behavior in this system. We have recently completed a draft genome assembly for this species, and discuss these results in a comparative genomic framework using available data from other social insect species.

Keywords:*genetics, genomics, behavior, eusociality, Halictidae*

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S512TH03

Genetics, Genomics and Evolutionary Entomology

Genomics of pheromone communication in honey bees

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A main objective in the study of social evolution is to understand how the social environment indirectly influences the expression of socially relevant traits. Social life influences many biological functions including reproduction, foraging and protection against predators. Such traits are controlled by the genotype of an individual as well as by social partners. In the honeybee, Apis mellifera, sociality relies on chemical communication to regulate several aspects of colony life. Caste specific pheromones are the hallmark of social insects. In the honeybee these pheromones regulate a plethora of behaviors, including the absolute reproductive dominance of the queen. The mandibular pheromone provides an excellent system for studying social communication processes at the gene level, in particular the expression of caste specific pheromones. To underline these, we performed a microarray study of the mandibular glands of queens, queenright (QR) workers and queenless (QL) workers. About 2554 genes were significantly expressed in the glands. The genes transcripts whose expression patterns were correlated with caste, revealed an overrepresentation of processes associated with oxidation-reduction activity, immune response, and several metabolic and biosynthetic processes. The genes transcripts whose expression patterns were significantly different between QL and QR workers (reflecting social environment), revealed an overrepresentation of genes associated with protein catabolism. In this study we make predictions about the biosynthetic steps and enzymes involved in the production of fatty acid based pheromones in the mandibular gland. Out of the 2554 significant regulated transcripts, we identified 32 genes that may play a role in pheromone biosynthesis.

Keywords:*Honeybee, Pheromones, Sociality, Genomics*

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100 million years of social evolution ? Lessons learned from seven ant genomes

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The genetic and genomic changes associated with the evolution, maintenance, and regulation of eusociality is currently not well understood in part because so far only one sociogenome (honeybee) was available. Building upon the recent sequencing of seven ant genomes we performed a comparative analysis of the genomic organization of two insect lineages that convergently evolved eusociality (bees and ants) and other solitary insects (Gadau et al. 2011). Ant genomes differ in many fundamental aspects from the honeybee genome , e.g. gene number, lineage specific genes, DNA methylation, miRNA composition, transcription factors, and transcription factor binding sites. Additionally, ant evolution over the past 10-100 million years has also lead to dramatic genomic reorganizations between the seven ants both in terms of gene composition and regulation.

Keywords:*social evolution*

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What makes a termite queen: Genes involved in maintaining reproductive division of labour

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Reproductive division of labour is the hallmark of insect sociality. It evolved in termites, as social cockroaches, independently from the social Hymenoptera. We study its molecular underpinning in a lower termite, Cryptotermes secundus. Here workers are totipotent and can explore all caste options: becoming a soldier, an inheriting replacement reproductive or a winged dispersing reproductive. With use of cDNA representational difference analysis, we identified five genes (Neofem1-5) which are significantly overexpressed in queens compared to workers. Applying a combination of RNA interference (RNAi), behavioural assays, endocrinological studies and GC MS analyses we tested two of these genes for their functional significance. We could show that inhibiting Neofem2 and Neofem4 makes workers behave as though the colony is queenless and that they are necessary for the queen to suppress worker reproduction. Neofem2's putative gene product suggests that it is a β-glycosidase. RNAi of Neofem4 also resulted in a change of the cuticular hydrocarbon profile of the treated queen, so that her smell was more worker-like. These results suggest that Neofem4 is involved in the production of the royalty scent that assures the reproductive monopoly of the queen.

Keywords:*social insects, termites, reproductive division of labour*

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Genomics of social behavior in fire ants

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The fire ant Solenopsis invicta is characterized by a remarkable form of social polymorphism, with the presence of one or several queens per colony being completely associated with allelic variation at a single Mendelian factor marked by the gene Gp-9. Because additional phenotypic differences in physiology, fecundity, and behavior are also completely associated with Gp-9, it has been hypothesized that the locus is actually comprised of multiple genes that form a supergene. To test this possibility, we mapped the monogyny/polygyne social polymorphism to higher resolution using restriction-site associated DNA (RAD) tag sequencing, which is a recently developed method that permits rapid SNP discovery and genotyping at low cost. By taking advantage of the recently completed draft genome sequence for fire ants, we were able to anchor many of the RAD tags to the genome, and we found that Gp-9 is associated with a non-recombining region covering ~13 Mb and corresponding to ~2/3 of the chromosome. Thus, variation in colony social form in fire ants is associated with a very large supergene which we propose to be a social chromosome.

Keywords:*fire ants, RADseq, social behavior, genomics*

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S512TH07

Genetics, Genomics and Evolutionary Entomology

Sociogenomics of conflict and cooperation in fire ant queens

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Founding behavior is a key feature of life history in social insects and one of the best examples of behavioral plasticity, since young queens can often adopt multiple strategies of colony foundation within the same species. Newly mated queens of the fire ant Solenopsis invicta can found new colonies alone (haplometrosis) or in group (pleometrosis). Here, the initial cooperation among queens, useful to defend the incipient colony against enemies and to increase the size of the colony more quickly, turns into conflict as soon as the first workers are close to emergence. Only one queen will be allowed to lead the mature colony while the others will die in fights with the dominant queen or as a consequence of the repeated aggressions performed by workers. We developed a microarray platform to monitor the expression of ~50K probes: these include a set of ~16K genes annotated from the Solenopsis invicta genome project, and a set of ESTs obtained from transcriptomic studies. We characterized the expression patterns in haplometrotic and pleometrotic queens. Counterintuitively, we found that the social context is more important than the outcome of the conflict: pleometrotic queens have very similar gene expression compared to haplometrotic queens. Moreover, changing the social status in pleometrotic queens from winner to loser and vice-versa triggers larger changes in gene expression than the social status itself. This means that the social environment is the major force driving gene expression in pleometrotic queens and its drastic manipulation produces effects at the genomic level.

Keywords:*fire ant, pleometrosis, queen, mating, founding behavior, genomic, microarray*

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Immunity in social insects: Insights from two Bombus genomes

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Group living offers considerable benefits but not without some obvious costs. In particular, high sociality comes with a greater risk of infection, especially when the groups are genetically homogenous, as in many social insects. Surprisingly, the first hymenopteran genome, the honeybee, revealed a reduced immune system relative to existing model insect species. Honeybees and many other highly social insects have complex, and highly successful behavioral defenses against parasites, which might compensate for this reduced immune repertoire. Since the sequencing of the honeybee genome, the solitary wasp Nasonia, a suit of ants, and now two bumblebee genomes have been sequenced which vary in complexity of sociality. We discuss immunity in bumblebees revealed by the two recently sequenced genomes in the context of sociality.

Keywords:*social insects*

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S513F01

Genetics, Genomics and Evolutionary Entomology

Insects as micro-scale biomass conversion systems and diversity of their lignocellulolytic enzymes

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Sizes of insects and their bioplans are generally suit for biomass processing. Their physical breaking-down systems, which consisted of the mandibles and the proventriculus of rigid cuticular structures, are ideal not only for herbivory but also for xylophage. The body sizes of xylophagous species allow them to dig into soft parts of wood avoiding highly lignified parts (heartwood and summer wood). The basic enzymatic digesting systems of xylophagous insects consist of endogenous enzymes mainly of GHF (glycoside-hydrolase family) 9 (hemimetaboly species), 5&45 (coleopteran species), although other family members have found as minor components including those of unknown functions. These endogenous enzymes are considered to digest surface area of exposed cellulose fibrils by the physical breaking of lignocellulose matrix to quickly supply physiologically required glucose of minimum amounts. For further comprehensive digestion of lignocellulose, many species, especially lower termites, combine a symbiotic digestive system in addition to the endogenous system (or seem to totally depend on ectosymbiotic cellulolytic fungi as is the case of Ambrosia beetles). The ligunocellulytic digestion of the termite symbiotic protozoa are considered as totally intracellular process isolated from the extracellular space, so mode of actions of possible synergistic effects with the endogenous enzymes are argued including presence/absence of such effects. In addition, the pretreating effect of possible endogenous proteins/substances of the termite salivary glands is also on the discussion with anecdotal/empirical evidences, which has not been reported with molecular proofs so far.

Keywords:*termite xylophagous cellulose cellase*

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A comprehensive dissection of lignocellulolytic enzymes in a wood-feeding cockroach, *Cryptocercus punctulatus*

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As fossil fuels are being depleted, much attention has turned to the most abundant carbohydrate energy resources on Earth, lignocelluloses, a naturally occurring complex of plant-derived materials that includes hydrophilic sugar polymers cellulose and hemicellulose, and a hydrophobic organic "glue"- lignin. Wood-feeding insects have intrigued scientists for decades because of their unique capability of breaking down the lignocellulose matrix of cellulose, hemicelluloses, and lignin in plant cell walls. The pressing needs for bioenergy and biomass conversion have redirected termite research to focus more on lignocellulose digestion and degradation. However, there is virtually no information available on the termite sister group, a wood-feeding cockroach punctulatus , which has more diversified cellulolytic symbionts than termites. To search for novel lignocellulolytic enzymes, we have initiated a tissue-specific metagenome-transcriptome sequencing project to inventory a comprehensive set of genes from both hosts and symbionts which potentially involved in the lignocellulose degradation as well as nitrogen recycling in the wood-feeding C. punctulatus. Such an effort would facilitate discovery and functional analysis of novel cellulolytic enzymes as well as provide sequence information on the nitrogen fixation by prokaryotic symbionts reside in the woodroach hindguts. These discoveries will be critical to breakthroughs in better understanding of nitrogen recycling in the ecosystem and potentially to improve the production of renewable energy through the discovery of novel cellulolytic enzymes that could contribute to the enzymatic pretreatment processes.

Keywords:*biofuel, biocatalyst, transcriptome, metatranscriptome, Cryptocercus punctulatus*

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Genetics, Genomics and Evolutionary Entomology

Screening, expression and characterizing novel cellulases and hemi-cellulases from metagenomic libraries of termite guts

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Ning Liua, Xing Yanb, Qianfu Wangb, Chanli Qianb, Lei Zhangb, Qian Wanga, Yongping Huanga, and Zhihua Zhoub§ aKey Laboratory of Insect Developmental and Evolutionary Biology; bKey Laboratory of Synthetic Biology, SIBS, CAS; Termites, as one of the most important wood-degrading organisms in the world, play an important role in lignocellulose degradation and carbon mineralization in natural ecosystems. Its intestinal microbial metagenome is believed to be a reservoir for new lignocellulases. Functional -based gene screenings of metagenomic libraries have been employed to obtain genes or gene clusters directly from environmental DNA. In our recent study, we combined a refined intestinal microbial DNA extraction method and multiple-displacement amplification (MDA) to obtain enough high quality and quantity of intestinal microbial DNA from termite guts. Two fosmid libraries were then constructed from the total microbial DNA isolated from the guts of a fungus-growing termite and a higher wood-feeding termite, respectively. Functional screening for endoglucanase, cellobiohydrolase, glucosidase and xylanase resulted in more than 400 positive clones. In total, about 200 of the positive ones were sequenced by the high throughput pyrosequence approach, and about 120 unique GH genes were annotated, the sequences of which were matched to the nearest GH genes in databases ranged from 29% to 76%. Fifty of the screened GH genes were expressed in E. coli and about 24 purified enzymes were obtained. The characteristics of the purified enzymes, including the optimal pH values and temperatures, the ranges of pH tolerance, thermostability and specific substrate activities, were investigated. § the presenting author

Keywords:*cellulase, termite, metagenomic library, displacement amplification*

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Integration of the lignocellulolytic systems of insects to biofuels industries

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Lignocellulose is the most abundant form of biomass on the planet and is a renewable resource that can serve as a feedstock for fuels and chemicals. It has been noted that the life systems with the function to efficiently degrade lignocellulosic biomass are very rare on the earth although many of the microbial systems (such as bacteria and fungi) would chronically degrade lignocellulosic biomass. However, after experiencing a long period of evolution, some wood-feeding insects have developed an incredible function to efficiently deconstruct lignocellulosic polymers from agricultural crops to forest woody substrates and rely on lignocellulosic biomass as their only foods. Cellulose digestion has been demonstrated in more than 20 families representing ten distinct insect orders, e. g. Thysanura, Plecoptera, Dictyoptera, Orthoptera, Isoptera, Coleoptera, Trichoptera, Hymenoptera, Phasmida, and Diptera. The ability of these insects to feed on wood, foliage and detritus has recently stimulated extensive investigations into the mechanisms of how these insects digest the structural and recalcitrant lignocellulose in their foods. This review addresses various lignocellulolytic systems, their potential values, challenges, and opportunities that exist for scientists and industries to advance the biofuel technology, where the following topics will be further addressed: 1) Scientific and industrial potentials of the natural biomass utilization systems; 2) Novel biocatalysts explored from natural biomass utilization systems and their engineering potential for industrial uses; 3) Novel microbial symbionts discovered from natural biomass utilization systems by "omics" technologies; 4) Bioreactor innovations mimicked and advanced from the efficient biomass utilization systems.

Keywords:*Cellulose-feeding insects, biofuels, lignocellulose, bioreactor,biorefining*

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Genetics, Genomics and Evolutionary Entomology

Digestion from within: Improving biomass feedstock through engineering multifunctional lignocellulose degrading enzymes using termite and wood roach lignocellulases

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The development of an enzyme-based biomass pretreatment system that would allow pretreatment under moderate temperatures and mild pH is attractive. However, biological processing of biomass requires a large number of lignocellulosic enzymes that are expensive to produce at industrial scales. One approach, which may simplify the pretreatment step, is to "digest from within" through the production of lignocellulosic enzymes in plant feedstock.Termites and their relatives, including the wood-eating cockroaches, can efficiently convert 95% of the recalcitrant lignocelluloses into fermentable sugars in their tiny bioreactor hindguts in less than 24 hours. Such efficiency and low energy inputs make the insect lignocellulases an ideal enzyme source for biomass pretreatment. Recently, we have applied genomic and proteomic tools to identify over 600 lignocellulase genes from the insect gut microfauna and functionally characterized several key genes in the lignocellulose digestion machinery. Previously, we have generated a number of chimeric lignocellulosic hydrolases that contain two to five functional modules joined by peptide linkers. These chimeras closely resemble the parental enzymes in kinetics and other enzymatic properties, and some exhibit improved synergy in degrading natural substrates when compared to mixtures of parental enzymes. Our overall hypothesisisplant feedstock can be optimized for pretreatment and saccharification through the "built-in" lignocellulases. Using the novel chimeric enzymes for this purpose is advantageous as they can simplify vector construction, downstream crop analysis and breeding.

Keywords:*Biomass Feedstock*

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Genetics, Genomics and Evolutionary Entomology

Profiling the mosquito transcriptome: Case studies of *Aedes aegypti* and *Anopheles gambiae*

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The understanding of gene expression profiles of organisms under defined conditions and stimuli offers the possibility to uncover the complex metabolic pathways and gene interactions contributing to the phenotype. Additionally, from a vector control perspective, this strategy allows the identification of: 1- key targets for monitoring the acquisition of a phenotype such as insecticide resistance; 2- cis-regulatory regions that could be co-opted to create transgenic pathogen-refractory insects. The recently-emerged RNA-seq technology was applied to study the expressional response of different strains of the dengue vector mosquito, Aedes aegypti following a blood meal and during dengue infection and the comparative transcriptomics of insecticide -resistant and -susceptible Anopheles gambiae mosquitoes.

Keywords:*Aedes aegypti, Anopheles gambiae, RNA-seq*

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Genetics, Genomics and Evolutionary Entomology

Application of functional genomics and transcriptomics to understanding lactation in the tsetse fly (*Glossina morsitans morsitans*)

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The tsetse fly is a unique insect and disease vector due to its unusual biology. The viviparous reproductive biology and lactation system the fly has developed is particularly interesting. In this work we combine the power of transcriptomic analysis by RNA-seq with the recently completed tsetse genome sequence to identify and annotate tsetse specific milk proteins, regulatory elements driving lactation specific gene expression and regulatory factors driving milk protein gene expression. RNA-seq analysis of lactating females versus non-lactating females allowed global identification of differential gene expression during pregnancy. This analysis confirmed the up regulation of previously identified milk proteins in lactating flies and identified a suite of novel tsetse milk proteins. The expression profile of all these genes is milk gland and larvigenesis specific. Transgenic analysis of the regulatory region for the major milk protein GmmMGP1 in Drosophila shows that this region drives female accessory gland specific gene expression during reproduction. In silico comparison of this regulatory region with the genome derived upstream regulatory regions of known and novel milk proteins identified conserved homeodomain type transcription factor binding site predictions. We identified 96 putative tsetse homeodomain proteins from the genome. Of the 96, 6 are differentially expressed in pregnant flies as predicted by the described RNA-seq data. Expression and tissue specific analysis of these six proteins identified a single homeodomain gene (Ladybird Late) as specific to the milk gland tissue. Knockdown of this factor resulted in an ~80% reduction of milk protein gene expression in pregnant females.

Keywords:*Functional Genomics, Transcriptomics*

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Genetics, Genomics and Evolutionary Entomology

Transcriptome profiling of sexual maturation and mating in the Mediterranean fruit fly, Ceratitis capitata

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Sexual maturation and mating in insects are generally accompanied by major physiological and behavioural changes. Many of these changes are related to the need to locate a mate and subsequently, in the case of females, to switch from mate searching to oviposition behaviour. The prodigious reproductive capacity of the Mediterranean fruit fly, Ceratitis capitata, is one of the factors that has led to its success as an invasive pest species. To identify the molecular changes related to maturation and mating status in male and female medfly, a microarray-based gene expression approach was used to compare the head transcriptomes of sexually immature, mature virgin, and mated individuals. Attention was focused on the changes in abundance of transcripts related to reproduction, behaviour, sensory perception of chemical stimulus, and immune system processes. Broad transcriptional changes were recorded during female maturation, while post-mating transcriptional changes in females were, by contrast, modest. In male medfly, transcriptional changes were consistent both during maturation and as a consequence of mating. Of particular note was the lack of the mating-induced immune responses that have been recorded for Drosophila melanogaster, that may be due to the different reproductive strategies of these species. This study, in addition to increasing our understanding of the molecular machinery behind maturation and mating in the medfly, has identified important gene targets that might be useful in the future management of this pest.

Keywords:*transcriptome, medfly, sexual maturation, microarray, mating*

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Cell death genes and their use in Tephritid pest control

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Pro-apoptotic proteins from the reaper, hid, grim (RHG) family are primary regulators of programmed cell death in Drosophila due to their antagonistic effect on inhibitor of apoptosis (IAP) proteins, thereby releasing IAP inhibition of caspases that effect apoptosis. Using a degenerate PCR approach to conserved domains from the 12 Drosophila species, we have identified the first reaper and hid orthologs from a tephritid, the Caribfly Anastrepha suspensa. As-hid is the first identified non-drosophilid homolog of hid, and As-rpr is the second non-drosophilid rpr homolog. Both genes share more than 50% amino acid sequence identity with their Drosophila homologs, suggesting that insect pro-apoptotic peptides may be more conserved than previously anticipated. In addition, these tephritid cell death genes could be used as functional and potent as cell death effectors, and to design improved transgenic lethality systems for insect population control.

Keywords:*programmed cell death, reaper, hid, Anastrepha suspensa, conditional lethality*

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Genetics, Genomics and Evolutionary Entomology

Genome annotation and functional genomics of insect olfactory binding proteins

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Olfaction plays a crucial role in insects' attraction to hosts and mates. Although the molecular basis of this interaction is not well understood it is likely that olfactory binding proteins (OBP) are involved in the first step of molecular recognition. We have defined OBP-specific sequence motifs based on highly conserved cysteine residues, developed an algorithm which has allowed us to identify genes encoding putative OBPs from several insect genome sequences and classified OBPs into Classic, Atypical, Plus-C, Minus-C and CSP subfamilies. Phylogenetic analyses showed a high conservation among Lepidoptera OBPs but a rapid expansion and high divergence of DipteraOBPs. Comparative analyses of OBP sequences reveal some mosquito-specific genes as well as conserved homologs with OBPs of other insects. The analyses of OBP-containing regions for microsynteny indicate a very high synteny between Aedesaegyptiand Anopheles gambiae. Our genome wide analysesof insectOBPs suggest that the rapid expansion and high divergence of DipteraOBPs have occurred probably by gene duplication after speciation. The genome of the parasitoid wasp Nasoniavitripennis contains the largest OBP family (90 members) so far reported in insects, and a new lineage specific domain arrangements, such as the double domain OBP subfamily of wasps and mosquitoes. qRT-PCR revealed that some OBP genes were highly transcribed in the antennae but also in other tissues. Expression of chemosensory proteins in the tsetse flyGlossinamorsitansmorsitans is related to female host-seeking behaviour.

Keywords:*odorant-binding protein, genome annotation, olfaction, chemical communication*

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The genome of *Clunio marinus* (Chironomidae, Diptera): A marine insect gives insights into circalunar and circadian clocks

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The marine midge Clunio marinus has precisely timed its life cycle to the tides by a combination of diurnal and lunar rhythms. Tidal regimes differ along the coast and local populations show corresponding genetic adaptations. Population genetic examination showed that the timing adaptations are of recent and common origin. Crossing experiments and QTL mapping revealed the loci underlying the timing adaptations, giving first insight in the genetics of circalunar clocks and their interaction with circadian clocks. In summary, Clunio is an exciting model for chronobiology and evolutionary genetics. We have now sequenced the Clunio marinus genome, using a genetic map as a backbone for genome assembly. The genome is <100 Mb in size and has ~70% AT content. Comparative sequencing of DNA pools from two field populations identified 586,422 SNPs, but only 1,359 (0.2%) of these are differentially fixed between populations. This underpins recent evolutionary divergence of populations and hints to which genes control functional differences, e.g. regarding the above-mentioned timing traits. Beyond, the genome sequence enables many other studies: Clunio is strikingly sexually dimorphic, females having reduced wings, legs, eyes and antennae. During secondary colonization of marine habitats Clunio has evolved water-repellent surfaces and tight osmoregulation. To date there is no other chironomid genome published. The closest sequenced relatives (Aedes, Culex, Anopheles) are ~220 million years divergent. Thus, the Clunio genome may aid the study of other chironomids (which are e.g. indicator species in ecotoxicology and climate reconstruction) and is a valuable resource for comparative insect genomics.

Keywords:*biological clock, lunar rhythm, reference genome, SNP, evolutionary genomics, comparative genomics, Chironomidae*

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S515F01

Genetics, Genomics and Evolutionary Entomology

The mode of action of dsRNA for control of western corn rootworm (*Diabrotica virgifera virgifera*) larvae

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Double-stranded RNA (dsRNA) is known to be effective in Diabrotica larvae via oral delivery. After ingestion, the dsRNA is thought to be taken up by the insect midgut cell and processed by the native RNAi machinery, which leads to specific knockdown of the target mRNA. The knockdown effect can cause lethality if the target mRNA encodes a protein with an essential function in the insect. Although processing of the dsRNA and target mRNA degradation appear to be conserved in western corn rootworm, some of the key steps in this process, such as uptake of dsRNA into midgut cells, are still unknown. In this presentation, we will present data on the dsRNA uptake mechanism and its selectivity for molecules of different sizes, as well as, the detailed morphological and cellular defects that lead to western corn rootworm (WCRW, Diabrotica virgifera virgifera) death upon ingestion of dsRNA targeting an essential gene.

Keywords:*RNAi, Diabrotica*

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Genetics, Genomics and Evolutionary Entomology

RNAi-aided baculovirus resistance in the transgenic silkworm: Application to sericulture

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Bombyx mori Nuclear Polyhedrosis Virus (BmNPV) infection is a major cause of silk cocoon crop losses in sub-tropical sericulture. Our earlier attempts to confer resistance to baculovirus infection by constructing silkworm transgenic lines carrying transgene encoding double stranded RNA for the essential viral gene, ie-1 proved rather efficient in preventing baculoviral infection (Kanginakudru et al. 2007 Insect Molecular Biology 16, 635-644). However, the transgenic lines were not robust enough to be exploitable in sericulture farms. We then constructed transgenic silkworms expressing short interfering RNAs (siRNAs) targeting several viral genes simultaneously, expecting that this would mount higher levels of baculoviral inhibition. Several transgenic lines, expressing siRNAs for four essential genes of the baculovirus, carrying transgenes expressing either sense (S), antisense (AS) or the double strand (DS) forms of the mosaic sequence were made. Very high level resistance to BmNPV was observed in the DS lines, as compared to the S, AS and their hybrid lines. Biochemical experiments suggested that the host-mediated RNAi machinery generates siRNAs in the transgenic lines. We then introgressed siRNA expressing transgene to the commercial but baculoviral susceptible Indian silkworm strain. Recurrent back-cross breeding coupled with microsatellite marker-aided selection led to the successful introgression of baculovirus resistance property to the high yielding strain. These transgenic lines are undergoing laboratory trials pending clearance from the Indian Govt for large scale field trials. This approach shows that the RNAi pathway can be successfully exploited in developing baculovirus resistant silkworm strains and that this could find commercial applications in sericulture.

Keywords:*RNAi, Baculovirus, Bombyx*

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Identification of fecundity related genes of the brown plant hopper through RNA interference

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The brown plant hopper, Nilaparvata lugens (Stal), is one of the most serious rice insect pests in many parts of Asia. After established a feeding-based RNAi method for this pest, it was used to identify functions of several fecundity related genes such as vitellogenin (Vg) gene, forkhead transcription factor (FoxA) and ecdysteroid receptor (EcR) gene. FoxA is required for ovarian mature in this insect. Rice-mediated RNAi experiments indicated that silencing of EcR gene decreased number of offspring by 34%-78%. RNASeq analyses showed that 1639 genes were up-regulated in 5th instar nymphae and 7408 genes in day 2 female adults between high and low fecundity populations. By using proteomics techniques, 39 and 54 different proteins were found in day 3 and day 6 female adults, respectively. Based on bioinformatics analyses of above findings, about 100 genes were selected for further RNAi studies. It is expected that about 10 fecundity related genes will be identified which may be potential target genes for controlling insect pests.

Keywords:*fecundity, RNAi, brown plant hopper, Nilaparvata lugens*

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RNAi for biological control

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The multicolored Asian ladybird beetle, Harmonia axyridis is a predator of numerous aphid species, and its use as a biological control agent has already been established. However, as adult beetles immediately tend to fly away from field crops, sustainable use in biological control has been restricted. To resolve this problem and make this biological control more efficient, a flightless strain that is unable to disperse from crops has been developed. We established a novel method to produce a flightless ladybird beetle for biological control using RNAi method which targets wing master genes. The injection of double-stranded RNA (dsRNA) for wing master genes into the larval body cavity of H. axyridis efficiently induced adult morphological defects that mimicked those of known loss-of-function phenotypes for these genes. As a consequence, wings were severely reduced and the ladybird beetles were flightless. The dsRNA injection did not have any obvious effect on the feeding behavior, and the voracious trait of H. axyridis remained unchanged. Thus, this RNAi method for flightless beetle production is very effective. As this RNAi method depends not on genetic transformation, but on posttranscriptional regulation, this biological control agent would be easily accepted by the public from an environmental and safety point of view. This technique opens sustainable use of flightless ladybird beetles in biological control and is widely applicable to other predators of pests for a biological control agent. Further, we should be able to design a "tailor-made insect" for ideal biological control using RNAi.

Keywords:*Harmonia axyridis, RNAi, biological control, wing master gene*

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RNAi of transcription factor BmPOUM2 for regulation of development of silkworm wing dics during metamorphosis

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The wing cuticle protein gene BmWCP4 is regulated by the transcription factor BmPOUM2, which binds to the promoter of the BmWCP4 gene and activate its pupa-specific expression during metamorphosis in Bombyx mori. Quantitative real-time PCR (qRT-PCR) and western blotting analyses indicate that silencing the gene coding for BmPOUM2 by RNAi during the wandering stage resulted in suppression of BmWCP4 expression and a failure of last instar larvae to complete larval-pupal metamorphosis. The epidermis in the thorax region became transparent and the wing discs growth and differentiation was arrested. The RNAi larvae failed to spin cocoons. qRT-PCR analysis showed that expression of other pupa-specific wing cuticle protein genes BmWCP1, BmWCP2, BmWCP3, BmWCP5, BmWCP6, BmWCP8 and BmWCP9, as well as dopa decarboxylase, and sericin-1, were also down-regulated by BmPOUM2 RNAi in the BmPOUM2 RNAi animals. These data suggest that BmPOUM2 is a critical important transcription factor for the larval to pupal transformation in B. mori and it is involved in the regulation of the pupal-specific expression of BmWCP4, probably other BmWCPs as well, during metamorphosis. This project is supported by grants from the National Basic Research Program of China (2012CB114602) and the Chinese National Natural Science Foundation (31172265).

Keywords:*RNAi, transcription factor, cuticle protein, metamorphosis, silkworm*

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Genetics, Genomics and Evolutionary Entomology

RNA interference to identify lethal genes involved in chitin metabolism of *Locusta migratoria*

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Chitin is widely distributed in epidermis and the peritrophic matrix of insects. The importance of chitin in insect development and the absence of chitin in vertebrates have led to a hypothesis of attacking insect chitin biosynthesis pathway as a novel target for developing safe and effective insecticides. The process of chitin biosynthesis and degradation consist of at least eleven key enzymes, but the roles of these enzymes in insect development have not been well elucidated. Five important genes putatively encoding trehalase 1 (TR1), glutamine: fructose-6-phosphate aminotransferase (GFAT), glucosamine-6-phosphate N-acetyltransferase (GNAT), UDP-N-acetylglucosamine pyrophosphorylase1 (UAP1) and chitin synthase 1 (CHS1). Have been identified to be involved in chitin biosynthesis in Locusta migratoria, In contrast, multiple chitinase genes and one β-N-acetylglucosaminidase gene have been identified to be involved in chitin degradation. By using RNA interference in different nymph stages, we observed that nymphs injected with dsRNA of GFAT, UDP1, CHS1, LmCht10 or LmNAG1 developed slowly, failed to detach from the old cuticle during molting process and showed high mortality. When the expression of LmCht10 was suppressed by RNAi, we found significantly reduced new cuticle development and cuticle thickness as compared with those of the dsGFP-injected controls. Our results suggest that GFAT, UDP1, CHS1, LmCht10 and LmNAG1 are essential for insect growth and development. The enzymes encoded by these genes could potentially serve as an excellent target for developing novel strategies for controlling this important pest.

Keywords:*RNAi, chitin metabolism enzymes, Locusta migratoria*

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Embryo lethal genes for pest control

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Trichogramma genus is one of the most commonly used parasitoids in biological control programs. Several studies have proven that T. brassicae is the most dominant with most distribution in different regions of Iran. Thelytoky system base on Wolbachia infection has observed in this species. As for role of this bacterium in induction of thelytoky and importance of application of thelytokous species in biological control programs this study was conducted. Studying of behavior was conducted under controlled condition: 25±1°C, 50±10 % RH and 16:8 L: D. Individual Trichogramma females were presented with egg patches consisting of 100 host eggs glued with water on to a 5×5 cm white cardboard square with different host quality(100 fresh and old host eggs) separately. The host eggs were separated by a distance of 5 mm. A sequence of six behavioral events was used to monitor host use by the Trichogramma females. Spent time for oviposition on each fresh egg was 75±0.36 S, while this amount on old eggs was 79±0.98 s. Observed acceptance time for each fresh egg was 17±0.54 s but this time on old eggs was 18 ± 0.86 s. Rejection time of each fresh egg was 10±0.43 s and this parameter was 11±0.67 for each old egg.

Keywords:*wolbachia, biological control, thelytoky, Tirchogramma brassicae*

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S601TU01

Behavior and Chemical Ecology

What plant-produced volatiles should we target to improve biological control?

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An increasing number of studies suggest that natural plant defenses can be manipulated and exploited for the control of insect pests. We have been studying a form of indirect plant defense, whereby, in response to insect feeding, plants emit various volatile compounds that attract the natural enemies of their attackers. For instance, after caterpillar feeding, maize plants emit a blend of volatiles that is highly attractive to parasitic wasps, but our efforts to find ways to alter the emissions to increase attraction of parasitoids has so far failed, mainly because attraction is determined by minor, as yet unidentified compounds. We have had more luck with belowground interactions, where insects-damaged roots release attractants for entomophatogenic nematodes. The roots of many varieties of maize release the sesquiterpene (E)-?-caryophyllene when damaged by larvae of the voracious western corn rootworm. Interestingly, most North-American maize varieties do not emit this signal, which significantly reduces nematode attraction and dramatically lowers nematode infection rates of pest larvae. We used genetic transformation to restore the signal in an American line and in field trials found that this drastically improved the plant's ability to attract beneficial nematodes. Hence, improving plant signals can be an ecologically sounds strategy to fight crop pests. However, a full understanding of the other roles that such compounds may play in multitrophic interactions is necessary before they can be safely employed in pest control.

Keywords:*tritrophic interactions, parasitoids, entomopathogenic nematodes, transgenic plants, pest control*

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Selecting volatiles in the field to protect brassicaceous crops against the cabbage root fly, *Delia radicum*

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Volatiles resulting from plant herbivore interactions play an important role in the behavioral decisions of phytophagous, predatory and parasitoid insects and could be used for managing pest insects. However, to date and after about 40 years of research, documented studies on applications in the field remain extremely scarce. Delia radicum, the cabbage root fly, is a major pest of brassicaceous crops for which classical control strategies are currently lacking. We have shown that dimethyl disulfide, a compound emitted by roots heavily infested by D. radicum larvae, was attractive for its main natural enemies and could lower the number of eggs laid on treated plants in the field by 60%. As a follow up of this work, we conducted another field study to select additional volatiles that could be used in a push-pull approach. Several synthetic HIPVs, selected on the basis of their potential action on the behavior of both the fly and its natural enemies, were placed in odor dispensers in experimental broccoli plots. Our results confirmed the role of dimethyl disulfide in reducing D. radicum egg numbers on broccoli plants and revealed other compounds that both influenced plant infestation by the fly and regulation by its main natural enemies. This study is a first step in designing a push-pull method to control the cabbage root fly but many steps need to be investigated before such a method can be implemented.

Keywords:*Herbivore-induced plant volatiles, conservation biological control, oviposition, predators, S*

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S601TU03

Behavior and Chemical Ecology

Induced plant semiochemical production to potentiate conservation biological control

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Rothamsted Research, United Kingdom

In mainstream crop protection by conventional pesticides, integration of biological control can be conflicted. However, as we strive to deliver crop protection more sustainably via the seed, we can deploy pest control chemistry using the principles of chemical ecology that allow us not only to interfere with pest development, but also to potentiate biological control agents. With criticisms mounting against release of exotic biological control agents and rising costs of inundation approaches, conservation biological control is more appealing and can now effectively be manipulated by defence-related plant chemistry, enhanced through molecular breeding or genetic manipulation. A further refinement is to effect this semiochemical enhancement of conservation biological control, when needed, by induction using natural plant activators for this purpose. New work on these approaches will be discussed and further novel opportunities proposed.

Keywords:*Conservation biological control*

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Developing new measures for rice pest management via chemical elicitor-based manipulation of volatile signals

Yong gen Lou
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Synthetic chemical elicitors of plant defense have been touted as a powerful means for sustainable crop protection. Yet, they have never been successfully applied to control insect pests in the field. We developed a high throughput chemical genetics screening system based on a herbivore-induced linalool synthase promoter fused to a GUS reporter construct to test synthetic compounds for their potential to induce rice defenses. We identified 2,4-dicholorophenoxyacetic acid (2,4-D), an auxin homologue and widely used herbicide in monocotyledonous crops, as a potent elicitor of rice defenses. Low doses of 2,4-D induced a strong defensive reaction upstream of the jasmonic acid (JA) and ethylene (ET) pathways, resulting in a marked increase of trypsin proteinase inhibitor activity and volatile production. Induced plants were more resistant to the striped stem borer Chilo suppressalis, but became highly attractive to the brown planthopper Nilaparvata lugens and its main egg parasitoid, Anugrus nilapavatae. In a field experiment, 2,4-D application turned rice plants into living traps for N. lugens by attracting parasitoids. Our findings demonstrate the potential of auxin homologues as defensive signals and show the potential of the herbicide to turn rice into a selective catch crop for an economically important pest.

Keywords:*rice, 2,4-dicholorophenoxyacetic acid, synthetic chemical elicitor, plant defense, tritrophic interaction*

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S601TU05

Behavior and Chemical Ecology

How to improve a crop plant's ability to recruit of bodyguards?

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Herbivorous arthropods are rarely pests in nature. However, in agriculture herbivorous arthropods cause significant losses. Our crops have been selected for a limited number of characteristics, especially related to product quality and quantity, in an environment where pests were eliminated by chemical control. Predators and parasitoids are important regulatory forces that limit herbivore populations below the economic injury level. To do so, these carnivorous bodyguards should be able to find their herbivorous victims at low densities. An important aid in finding their victims is to use herbivore-induced plant volatiles (HIPV). Although our crops have never been selected for the emission of HIPV they do emit these cues in response to herbivore infestation. Cultivars within a crop vary in the emission of HIPV and in the degree of attraction of carnivorous arthropods. This allows for the selection of cultivars that collaborate best with biological control agents. Alternatively, plant treatments may also influence the emission of HIPV and thus the attraction of bodyguards. In this presentation I will identify options to exploit knowledge on HIPV to improve a crop's ability to recruit its bodyguards.

Keywords:*biological control - bodyguard - predator - parasitoid*

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Biological control using plant volatiles that attract specialist parasitic wasps of target pests

Masayoshi Uefune[1], Junji Takabayashi[2]
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Plants emit so-called herbivore-induced plant volatiles (HIPVs) that attract carnivorous natural enemies of herbivores. We already reported that the synthetic blend of diamondback moth (Plutella xylostella) (DBM) larvae-induced cabbage plant volatiles attracted the specialist parasitic wasps Cotesia vestalis under both laboratory and field conditions. We show here that by setting the synthetic blend in greenhouses in a countryside agricultural field, we decreased the number of DBM larvae and increased the number of C. vestalis in greenhouses. We will discuss the possible use of synthetic blend of HIPVs for pest control.

Keywords:*plant volatiles, diamondback moth, Cotesia vestalis, pest control*

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Manipulation of belowground scents for enhanced plant protection

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Insect herbivory induces the synthesis and release of specific volatile compounds in plants. These volatiles have been shown to be highly attractive to natural enemies of the herbivores, such as predators, parasitic wasps or entomopathogenic nematodes. Few key compounds mediating these so-called tritrophic interactions are known, but in most cases they remain to be identified. In maize, the volatiles emitted upon feeding by leaf- or root feeding arthropod herbivores have been particularly well studied in the laboratory. Moreover, several genes and biochemical pathways responsible for the production of the emitted volatiles have been elucidated and described. These advances in understanding the volatile emissions and their ecological significance open up novel ways to modify plant volatile blends in order to enhance their attractiveness to natural enemies. Based on our experience, we present here an overview of the most promising results in manipulating the emission of specific volatile compounds, while simultaneously enhancing the responsiveness of natural enemies to particular compounds to enhance biological control of belowground herbivores of maize. However, such manipulative strategies require great caution and we emphasize the importance of taking a holistic ecological approach and to precisely evaluate risks and benefits of modifying volatile blends in the context of pest management.

Keywords:*belowground chemical ecology, entomopathogenic nematode, pest control, plant defense*

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Relative importance of host-derived and plant-derived volatiles in the attraction of the larval parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*

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By utilizing a six-arm olfactometer, we compared the responses of the two larval endoparasitoids, Cotesia marginiventris and Microplitis rufiventris, to the odors of maize plants that were induced to emit volatiles. Naive wasps and wasps that had had an previous oviposition experience on an host-damaged maize plant were offered the choice between undamaged maize plants, undamaged plants treated with caterpillar regurgitant, freshly damaged plants (scratched and treated with caterpillar regurgitant), plants with the same but older damage, plants with old and a fresh damage and plants actively fed on by herbivores. The results revealed that naive C. marginiventris are highly responsive to freshly damaged plants (which typically release green leafy volatiles) and become more responsive to plants with older damage that release specific herbivore-induced volatiles after experience. In contrast, naive M. rufiventris respond best to the complete blend of plant-produced volatiles, while experience rendered this wasp more responsive to volatiles that are directly associated with the herbivores and/or their by-products. Although both wasps have similar host plant ranges, they clearly employ different strategies to find their hosts.

Keywords:*herbivore-induced plant volatiles, indirect defense, natural enemies*

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S601TU10

Behavior and Chemical Ecology

Effects of inter- and intra-specific plant volatile interactions on aphids and ladybirds

Velemir Ninkovic[1], Inger Ahman[2], Iris Dahlin[3], Martin kellner[4], Robert Glinwood[5]

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In recent years it has become more and more apparent that chemical interactions between undamaged plants are very complex and highly dependent on genotype and species. These chemical interactions are expressed via volatile substances and have affects across three trophic levels; they affect barley biomass allocation, aphid settling responses and searching behaviour of predatory ladybirds. However, the effects of plant volatile communication on aphids and ladybirds depend both on the volatile emitter and receiver. Interspecific interactions between barley and certain weed species have also been found to be species dependent. In a recent study it was found that older barley cultivars were generally better volatile receivers while younger barley cultivars were more likely to provoke a response in other cultivars. Although certain cultivars respond to volatiles from other cultivars more frequently than others, the responses are specific to certain cultivar combinations. This indicates that the interaction between the emitting and receiving cultivar works in a key-lock fashion and the exact dynamics of this interplay are still to be discovered. The results stimulate discussion on plant responses to their environment as an advanced capacity of a plant to adapt to current conditions. It may be hypothesized that herbivores closely adapted to plant physiology are more sensitive to plant responses to volatiles, and that the third trophic level (predators of herbivores) respond to traits in the host plant that are shared with stress conditions

Keywords:*aphids, ladibitrds, barley, tri-trophic interactions, volatile plant interactions*

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S601TU11

Behavior and Chemical Ecology

Identification of volatile compounds emitted by *Artemisia ordosica* and changes due to mechanical damage and weevil-infestation

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Volatiles emitted by healthy, mechanically damaged and weevil-infested Artemisia ordosica were obtained through dynamic headspace method and analysed by automatic thermal desorption-gas chromatography-mass spectrometry (ATD-GC-MS). Twenty-eight compounds in all were identified and the qualitative as well as quantitative differences were compared. The green leaf volatiles 2-hexenal, (Z)-3-hexen-1-ol, 2-hexen-1-ol, 1-hexanol and (Z)-3-hexen-1-ol, acetate were present in all of the damaged plants, but in relatively lower proportions when plants infested by weevil Adosopius sp.; while terpenoids α-copaene, β-cedrene, (E, E)-α-farnesene and esters methyl salicylate were only present in weevil-damaged plants. The volatiles from healthy and weevil-infested leaves were dominated by D-limonene, whereas mechanical damages emitted β-pinene as the dominant compound.

Keywords:*Artemisia ordosica, Artemisia, Asteraceae, Adosopius sp., healthy, mechanically damaged, weevil-dama*

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Inducible responses to herbivory affect diamondback moth oviposition patterns and negatively affect larval predation.

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Neonate diamondback moth, Plutella xylostella L (Lepidoptera: Plutellidae), larvae feed on leaves young of their Brassicaceous host plants. Within intact cabbage (Brassica oleracea L. Capitata, cv. sugarloaf) plants eggs are preferentially laid on the lower plant parts but in herbivore-damaged plants eggs are preferentially laid on the upper leaves, close to larval feeding sites. However, within Chinese cabbage (Brassica rapa L. Pekinensis, cv. Wombok) plants eggs are preferentially laid on upper leaves in both intact and herbivore-damaged plants, although intact leaves are preferred over herbivore-damaged leaves. The effects of these responses on the susceptibility of larvae to predation on cabbage plants were tested in laboratory and field studies. In the laboratory, larvae hatching from eggs laid on the lower leaves of non-induced cabbage plants took longer to establish feeding sites and were significantly more susceptible to predation than larvae hatching from eggs laid close to larval feeding sites on herbivore-induced plants. This indicates that inducible responses to herbivory can increase herbivore survival in the presence of predators. In field studies predators caused significant larval mortality but oviposition site did not affect neonate survival. However, oviposition site did affect diamondback moth survival during periods of heavy rainfall and survival rates were greater when eggs were laid on the lower leaves of non-induced plants. The study demonstrates the complex interactions between the herbivore, its host plants and its natural enemies and provides a basis from which we can begin to understand observed inter- and intra-plant distributions of diamondback moth in Brassica crops.

Keywords:*Brassica crops, Plutella xylostella, predators, abiotic mortality, biotic mortality*

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The chemical heterogeneity of plant defence is exploited by insects to avoid harm and find safe havens

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Insects are sophisticated animals and many have evolved physiological adaptations that negate induced plant defenses. Plants have evolved sensitive ways to detect herbivores, and interrelated strategies to defend themselves. Insect herbivory induces synthesis of the phytohormone jasmonic acid, which activates downstream metabolic pathways for various biochemical defenses such as toxins and digestion inhibitors. By visualizing jasmonic acid-responsive gene expression within whole plants, we have uncovered short-term limits to the systemic spread of plant chemical defense. By carefully tracking these, along with insect movement, we have found there are plant-wide signals early in the induction process that allow insects to detect the heterogeneity of plant chemical defences. Vulnerable insects can use these signals to move around the plant, avoiding localised sites of induction and staying ahead of induced toxic metabolites. Avoiding induced tissue in this way would provide time for insect physiological adaptations to defensive metabolites to develop. Previously, we have found that 1st instar H. armigera spend less time feeding and more time moving than 3rd instars, but despite this, 1st instars have a higher relative growth rate. Behavioural resistance to induced defenses would be expected to benefit physiologically-vulnerable insects more than those accustomed to the changed conditions. Hence, it seems likely that, as physiological adaption develops along with aging of the larvae, younger instars would be the most likely to evolve behaviour in response to induced plant defense.

Keywords:*Helicoverpa armigera, jasmonic acid, Arabidopsis, insect resistance, plant defense, heterogeneity*

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Using transgenic Arabidopsis plants containing a luciferase reporter gene to map the spatial and temporal expression of genes involved in plant defence against herbivory.

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Plants have evolved sensitive ways to detect herbivores, and interrelated strategies to defend themselves. Insect herbivory induces synthesis of the phytohormone jasmonic acid, which activates downstream metabolic pathways for various biochemical defences such as toxins and digestion inhibitors. A limited number of genes involved in induced defence pathways of the model plant Arabidopsis have been identified. We have used transgenic Arabidopsis containing the gene for firefly luciferase (Luc) as a reporter fused to promoters of defence genes of interest to study the spatial and temporal pattern of defensive gene expression in plants. Luciferase catalyses a bioluminescent reaction and is a sensitive reporter gene for the real-time, non-invasive imaging of gene expression in planta using photon-counting cameras. Success using this technique requires careful attention to technical aspects such as penetration and pre-equilibration of the substrate luciferin into plant tissue. We have visualised the defence response in plants elicited by the application of methyl jasmonate, and by herbivory of a generalist- and specialist- feeding insect. We have uncovered short-term limits to the spread of defensive gene expression from the site of elicitation, but also systemic expression of induced genes in the longer term. To date we have studied plant response to limited herbivory, i.e. one to a few feeding bouts. Overall, the pattern of expression in response to this limited elicitation is one of spatial patchiness and temporal transience resulting in a heterogeneously defended plant at any given point in time. Currently we are investigating plant defence in response to extended herbivory.

Keywords:*herbivory, induced plant defence, luciferase transgenes*

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S602TU04

Behavior and Chemical Ecology

Salicylic acid signaling plays an important role in mediating indirect plant defense against whitefly

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Herbivore attack is known to induce indirect plant defense by releasing of volatile compounds that attract natural enemies of the herbivore. However, little is understood about the underlying mechanisms involved in indirect defense induced by the phloem-feeding insect, such as whitefly and aphid. Here, we investigate the indirect plant defense of Arabidopsis thaliana against the whitefly Bemisia tabaci, i.e. the attraction of the parasitoid wasp Encarsia formosa, as well as involved signal-transduction pathway. Parasitoids E. formosa were significantly attracted by the odors emitted from wild-type (WT) plants infested with B. tabaci compared to uninfested WT plants. This attraction is shown to result from the enhancement in ?-myrcene emission from WT plants infested with B. tabaci. Exogenous salicylic acid (SA) application also led to the attraction of parasitoids, but not exogenous jasmonic acid (JA). Remarkably, the attraction of parasitoids still occurred in uninfested SA-elevated (cpr-6), infested JA-deficient (dde2-2), infested ethylene-insensitive (ein2-1) mutants, but not in infested SA-defective mutants (NahG and npr-1). Taken together, we conclude that the SA signaling pathway plays a key role in mediating whitefly-induced indirect plant defense.

Keywords:*Indirect plant defense, phloem-feeding insect, salicylic acid, signaling pathway*

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S602TU05

Behavior and Chemical Ecology

Effects of tomato plants responses induced by *Frankliniella occidentalis* on the behavioural response and host preference of *Bemisia tabaci*

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The differences of the host preference and behavioural responses of B. tabaci on different treatments (undamaged plants (CK) vs plants damaged by B. tabaci (B); undamaged plants (CK) vs plants damaged by the F. occidentali (F); plants damaged by B. tabaci plants(B) vs damaged by B. tabaci and F. occidentalis (B+F)), were studied with the methods of Y-tube olfactometer and choice test. And the differences of the volatiles of different treatments were analyzed by GC-MS. The behavioural responses of female adult B. tabaci to different treatments in CK vs B and CK vs F treatment combinations were not significantly respectively. But in B vs B+F treatment combination, the female adult B. tabaci were attracted to the plant damaged by the B. tabaci. In the choice test of CK vs B and CK vs F, the adult B. tabaci prefered to feed and oviposit on the plant undamaged and in B vs B+F, the adult B. tabaci prefered the plant damaged only by B. tabaci and also prefered to oviposit on it. Furtherly, the analysis of the volatiles of different treatments showed that Comparing to the host plant damaged by B. tabaci, the amount of β-myrcene which shows strong attractableness to B.tabaci, reduced significantly and the amount of β-phellandrene which shows repellence to B. tabaci, increased significant in the plants damaged by both herbivores. Taken together, the plant responses induced by F. occidentalis have a great effect upon the host preference and behavioural responses of B. tabaci.

Keywords:*Frankliniella occidentalis, Bemisia tabaci, induced plant response, behavioral responses, host prefe*

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Soil microbial community and plant-insect interactions

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The soil microbial community may have a profound effect on the biochemical, physiological and morphological aspects of plants. Plant Growth Promoting Rhizobacteria (PGPRs) and Arbuscular Mycorrhizal Fungi (AMF) are important components of soil microbial community that are being commercially exploited for plant growth promotion, biological control and induction of plant systemic resistance against diverse pathogens. However, their role as biological pest control agents remains virtually unexplored. The aim of this study is to determine whether soil microbial community affects herbivore attack above ground. Experiments will be presented in which the effects of different soil microbial enhancement methods are tested on several crops and the consequences for above ground insects determined.

Keywords:*Soil microbial community, Plant-insect interactions, Multitrophic interactions*

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Whitefly infestation elicits defense responses against bacterial pathogens on the leaf and root and belowground dynamic change of microflora in pepper

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Plants have evolved general and specific defense mechanisms to protect themselves from diverse enemies, including herbivores and pathogens. To maintain fitness in the presence of enemies, plant defense mechanisms are aimed at inducing systemic resistance: in response to the attack of pathogens or herbivores, plants initiate extensive changes in gene expression to activate "systemic acquired resistance" against pathogens and "indirect defense" against herbivores. Recent our work revealed that leaf infestation by whiteflies, stimulated systemic defenses against both an airborne pathogen and a soil-borne pathogen, which was confirmed by the detection of the systemic expression of pathogenesis-related genes in response to salicylic acid and jasmonic acid-signaling pathway activation. Further investigation revealed that plants use self-protection mechanisms against subsequent herbivore attacks by recruiting beneficial microorganisms called plant growth-promoting rhizobacteria/fungi, which are capable of reducing whitefly populations. Our results provide new evidence that plant-mediated aboveground to belowground communication and vice versa are more common than expected.

Keywords:*aboveground, belowground, PGPR, Ralstonia solanacearum, whitefly, Xanthomonas axonopodis*

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S603W01

Behavior and Chemical Ecology

The chemical ecology involved in cuckoo bumblebee offspring survival in host nest

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In social insects, the high energetic costs of brood care have promoted the evolution of social parasites that exploit worker force of conspecifics or heterospecifics. In bumblebees, all the species of the subgenus Psithyrus have lost their worker caste and are completely dependent on host workers to produce their offspring. One of the most striking challenges for these social parasites is to avoid the detection and aggression by their hosts. Many studies have shown how socially parasitic queens overcome host recognition systems to enter successfully into host colonies. However, once a social parasite has successfully usurped a host nest, its emerging offspring still face the same challenge of avoiding host recognition. This is especially the case for the young Psithyrus males who possess a strong chemical signature because of their early production of species specific sex pheromones. Therefore, host workers might be able to recognize them. We hypothesized that parasitic males should require a strategy to prevent host agonistic behaviors. This study presents how males of the cuckoo bumblebee Bombus vestalis fool Bombus terrestris workers during their intranidal life. Our results show that parasitic males produce an allomone that repel attacking host workers. This is the first example of an active exfiltration strategy in social parasites.

Keywords:*Social Parasite, Psithyrus, Repellent Allomone*

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Feeding and oviposition preference of *Frankliniella occidentalis* for crop and weed species commonly occurring in French bean fields in Kenya

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Western flower thrips (WFT), Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), is an important pest of French beans in Kenya. However, information on the feeding activity and oviposition preference of WFT on crop and weed hosts associated with French beans in Kenya is lacking. To determine the feeding and oviposition preference of WFT for crop and weed plants commonly encountered in French bean fields in Kenya, no-choice and choice experiments were conducted using four important crop and weed plants. Among the crop plants tested, highest feeding and oviposition activity of WFT was recorded on courgette/zucchini (Cucurbita pepo L.) and French beans (Phaseolus vulgaris L.). Spinach beet (Beta vulgaris L.) and sweet pepper (Capsicum annuum L.), were of minor importance for feeding and oviposition. Among the weeds tested, gallant soldier (Galinsoga parviflora Cav.) was the most preferred host plant for feeding and oviposition compared to Chinese lantern (Nicandra physaloides L.), wild crucifer (Erucastrum arabicum Fisch. & C.A. Mey.) and pigweed (Amaranthus hybridus L.). Nevertheless Phaseolus vulgaris was the most preferred host for feeding and oviposition in presence of the weed species G. parviflora, E. arabicum and A. hybridus. A positive correlation between the number of feeding punctures and the number of eggs oviposited by WFT on crop and weed plants was observed. The results of this study show that P. vulgaris, C. pepo and G. parviflora are both good feeding and oviposition hosts of WFT. Cucurbita pepo and G. parviflora may serve as potential sources of WFT outbreaks within French bean fields.

Keywords:*western flower thrips, choice test, host, no-choice test*

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Responses of *Riptortus pedestris* (Hemiptera: Alydidae) to host food and of *Apolygus spinolae* (Hemiptera: Miridae) to host volatiles

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For many insect species, host plant plays an important role on their communications, and thus on the formation of feeding and mating aggregations, on insect strategies to locate both hosts and mates. Two different types of hosts, sweet (non-astringent) persimmon and soybean, can affect the pheromone production and reproduction of Riptortus pedestris male and female, respectively. Male adults fed on soybean produced all kinds of pheromone components, (E)-2-hexenyl (Z)-3-hexenoate, (E)-2-hexenyl-(E)-2-hexenoate, tetradecyl isobutyrate, and octadecyl isobutyrate. Female adults fed on soybean could normally lay eggs. However, males fed on sweet persimmon did not produce octadecyl isobutyrate and especially, tetradecyl isobutyrate which was known as a key functional component of its pheromone. No females fed on sweet persimmon could lay eggs. This finding indicates that host resources greatly affect the chemical communication and reproduction of R. pedestris. The green pale plant bug, Apolygus spinolae, has been known as a grape, sweet persimmon and green tea pest feeding on young leaves. Responses of the plant bug were tested to the several compounds emitted from green tea leaf, grape vine, and from the plant bug itself, using electroantennogram (EAG) and in the fields. EAG responses of A. spinolae female adults were recorded using 11 volatile compounds. Field attractiveness tests for both female and male were done using two more compounds in 2010 and 2011. Through these experiments, two compounds, methyl salicylate and tridecane were selected as possible attractants for A. spinolae.

Keywords:*Riptortus pedestris, Aploygus spinolae, EAG, host, tetradecyl isobutyrate*

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Study on maize pollen volatile compounds as potential attractant to blood fed and unfed female *An. arabiensis* under laboratory

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This study on main Ethiopian vector of malaria, Anopheles arabiensis was designed to identify potential attractant volatile compounds from two differentially attractive strains of maize pollen, BH-660 (Bako Hybrid-660) and ZM-521 (Melkassa 2). The behavioral responses demonstrated by blood fed and non-blood fed adult female An. arabiensis to pollen and extracts from BH-660 and ZM-521 illustrated olfactory mediated attraction to both strains of maize pollen. The An. arabiensis were attracted to pollen and extracts from both strains differently: blood fed mosquitoes were attracted to lower doses of BH-660 than to ZM-521 volatile for extracts and pollens; whereas unfed females were attracted to lower doses of ZM-521 than to BH660 maize pollen and odor extracts. In the same line, the pollen and extracts of BH-660 maize were more attractant than the other maize va ZM-521 maize variety. Importantly, the outcome of the study implies that olfactory cues were the driving external factor that assisted both blood fed and non-blood fed An. arabiensis to inhabit areas near BH-660 and ZM-521 maize pollen.

Keywords:*An. arabiensis, attractant, bioassay, BH-660, extract, ZM-521*

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Attraction of female *Anopheles stephensi* mosquitoes to the spores of entomopathogenic and non-entomopathogenic fungi

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We performed behavioral Y-tube olfactometer assays in the laboratory to test the upwind flight responses of the mosquito, Anopheles stephensi a major vector of human malaria in Asia, toward the fungal spores of two entomopathogenic species, Beauveria bassiana and Metarhizium acridum. Our group has been working toward the goal of applying spores to various indoor surfaces of dwellings as a biopesticide. Unfed female An. stephensi mosquitoes were tested one-at-a-time for their upwind flight propensity toward the arm of the Y-tube that contained a 50 mg loading of spores, versus the other arm that contained a blank filter paper. The females clearly exhibited a strong attraction towards B. bassiana spores; 75% of the females tested flew upwind to within 1 cm of the spores, and a significant percentage of these actually landed on the spores themselves. A high percentage of the mosquitoes (70%) was likewise attracted to 50 mg of the spores of M. acridum, and also to a lesser degree (65%) to 50 mg of the spores of Aspergilus spp., a non-entomopathogenic species. The spores of Penicilium spp., on the other hand, were deterrent to An. stephensi females, 80% of which preferred to fly up the blank arm of the Y-tube olfactometer and not to the Penicilium spores. In competitive choice-tests between the spores of different fungal species, those of B. bassiana were preferred, with 67%, 67% and 82% flying upwind to these spores vs. those of M. acridum, Aspergillus spp and Penicillium spp , respectively.

Keywords:*Anopheles stephensi*

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GC-EAD analyses of phytophagous insects to volatiles from different host plants

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The differences in chemical profiles of volatiles, especially those semiochemicals detected by insect antennae, are predicted to play important roles in mediating host preference of phytophagous insects. To test the above protection, two model systems were utilized for investigation of plant volatiles involved in host preference. The first one includes the giant swallowtail butterfly, Papilio cresphontes (Lepidoptera: Papilionidae), and its two key host plants, Zanthoxylum clava-herculis, and Ptelea trifoliata, and a non-preferred host plant, Sassafras albidum. The second one includes the long-legged chafer, Hoplia spectabilis, a preferred host plant, Hippophae neurocarpa, and two non-preferred host plants, Myricaria germanica, Potentilla fruticosa. Preliminary GC-EAD tests with P. cresphontes revealed four components each from Z. clava-herculis and P. trifoliata (three peaks common to both extracts) and seven from S. albidum (one shared with Z. clava-herculis) which elicited GC-EAD activity in P. cresphontes females. GC-EAD tests H. spectabilis revealed that at least ten components from the three plants elicited GC-EAD activity in both sexes of H. spectabilis, which were identified as trans-2-hexenal, cis-3-hexenylacetate, cis-3-hexenol, nonanal, decanal, (E,Z)-2,6-nonadienal, citral, methyl salicylate, benzylalcohol, phenol, eugenol. The first four components and an unidentified component in trace amount were shared by all three host plants. Plant derived volatile compounds shared by the tested plants may be utilized by herbivores for host plant selection. Future studies will include examination of non-host plant species by GC-EAD and GC?MS analyses to compare active components among various host plants that may contribute to host-plant preference.

Keywords:*Plant volatile, Papilio cresphontes Cramer, Hoplia spectabilis Medvedev, GC-EAD, GC-MS, host preference*

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Olfactory receptor neurons and host specificity in phytophagous insects

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Phytophagous insects use olfactory information to locate their host plants. Therefore, their olfactory sensory system is directly related to the volatile emanations of their host plants. Here we show the relationship between the response profiles of olfactory receptor neurons (ORNs) and the profiles of volatile compounds of host and non-host plants in two weevil and four moth species. We identified ORNs in the weevils Sitona lepidus and S. discoideus and the moths Cydia pomonella, C. succedana, Epiphyas postvittana and Uraba lugens, by using single sensillum recording technique. Each species shows distinct species-specific set of ORN populations each of which has unique response spectrum to specific range of plant volatile compounds. The plant-volatile sensitive ORN populations could be classified into either agonist or antagonist, depending on their responsiveness and the presence of their active compounds in the host and non-host plants. We hypothesize that the phytophagous insects use combinational sensory input of both agonist and antagonist ORNs for locating their host plants, and the presence of antagonist ORNs enhance the discriminating ability between host and non-host plants. Based on this, it would be possible to predict the range of host plants for invasive insect species by comparing their ORN profiles and the chemical profiles of volatile emanations of plants. Identifying agonist and antagonist ORNs can also provide critical information in developing attractants and repellents.

Keywords:*host location, moth, olfactory receptor neuron, plant volatiles, single sensillum recording, weevil*

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Response of female *Bactocera papayae* (Diptera: Tephritidae) on five mango varieties

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Fruit fly is one of key pest on fruits, the damage on fruits can reach 100%. The objective of this research was to study the respons of female B. papayae on 5 mangoes varieties. Tests conducted on five varieties of mango (Gedong, Arumanis, Cengkir, Bapang and TO) at different levels of maturity of mango (young fruit, mature / ripe and full ripe) using a modified Olfaktometer tube. Each treatment is infested of 50 female fruit flies are placed in the middle of the tube. At each end of the Olfaktometer tube is placed one variety of mango. Observation was done on the attractiveness of fruit fly oviposition sites compare with the different character of each mango varieties. Response of B. papayae on the mango varieties, indicating that the fully ripe mangoes more chosen by female fruit flies (83.6%), ripe mango (52%) and young mango (32.4%). Morphological characters of the full ripe mango, has a soft exopericarp and high sugar levels. Gedong varieties more chosen by female fruit flies for oviposition, compared with other varieties. Strong scent of varieties gedong, became one of the factors that influence the choice of emission levels, of volatile compounds released by the varieties Gedong allegedly more powerful than the other varieties. The results of regression analysis and correlation, suggesting that the aroma was positively correlated and have significant influence in the selection of the host. This suggests that the aroma plays an important role in the selection of the host.

Keywords:*Response, Bactrocera papayae, mango, oviposition*

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S603W09

Behavior and Chemical Ecology

Fatty acid amino acid conjugates diversification in lepidopteran caterpillars and physiological adaptation found in plants to the FACs.

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Fatty acid-amino acid conjugates (FACs), first identified from beet armyworm Spodoptera exigua larvae (Alborn et al., 1997), have been found in noctuid as well as sphingid caterpillar oral secretions and especially volicitin [N-(17-hydroxylinolenoyl)-L-glutamine, 17OH-volicitn] and its biochemical precursor, N-linolenoyl-L-glutamine, are known elicitors of induced volatile emissions in corn plants. We conducted FAC screening of 29 lepidopteran species and found FACs in 19 of these species (Yoshinaga et al., 2010). Thus, FACs are quite commonly synthesized through a broad range of lepidopteran caterpillars. Recently, we have newly identified N-(18-hydroxylinolenoyl)-L-glutamine (18OH-volicitin) from Manduca sexta regurgitants and showed that 18OH-volicitin was no more active than N-linolenoyl-L-glutamine in corn seedlings while 18OH-volicitin was as active as 17OH-volicitin when treated on solanaceous plants such as tobacco or egg plants, host plants of M. sexta. Meanwhile, these glutamine type FACs including 18OH-volicitin are infinitesimal components in M. sexta. Instead the larvae synthesize large amounts of glutamic acid type FACs as main components, which show half of the activity in corn plants and the almost same activity in egg plants. These results suggest that plants have developed the defense system customized to their pest insects.

Keywords:*insect-plant interaction, fatty acid-amino acid conjugates, elicitors*

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S603W10

Behavior and Chemical Ecology

Gall aphids manipulate plant volatiles for defence against vertebrates

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Gall-forming insects manipulate the structure and function of their host plant to gain protection and better nutrition. Since gall traits are controlled by the insect, galls are considered as their extended phenotype. Little is known about the metabolism and function of plant volatiles in galls. Around the Mediterranean, several aphid species (Fordinae) induce typical galls on Pistacia spp trees. Field observations suggested that natural enemies, such as birds, goats and herbivorous insects are deterred by the galls. The aphid Slavum wertheimae induces conspicuous large, cauliflower-shaped galls on the lateral buds of P. atlantica trees. We hypothesized that gall volatiles may act as warning (deterring) signals. Comparative analyses of defensive secondary metabolites revealed that, compared with leaves and fruits, the galls are heavily protected with higher levels of tannins, mono- and sesquiterpenes. Furthermore, galls released large amounts of volatile terpenes, which increased by 80% following mechanical damage. In bioassays we found that galls are indeed unpalatable and that scent is sufficient to deter both goats and birds; suggesting a signaling role for volatiles in the gall's headspace. We conclude that the aphids are well protected by the secondary metabolites in the galled tissue while volatiles serve in the front line of gall (insect) defenses. Volatiles in the headspace of galls are the external expression of the aphid's extended phenotype.

Keywords:*plant defence, herbivore defence, volatiles, terpenes*

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Non-host volatiles to control bark beetles - A new forest protection method under development

Carl Rikard Unelius
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After storm events there is a need to protect the forest. Storm felling creates fresh stand edges with trees stressed due to the quick change of environmental conditions (e.g. sun exposure) and thereby susceptibility for bark beetle attack. Newly exposed stand-edges are in need of stabilisation against the outbreak of bark beetles that inevitable will occur in the years after a big storm. In recent years attempts have been made to use natural non-host volatiles (NHV) from broad-leafed trees to protect conifer trees against bark beetles. In this talk recent advances in the development of this method will be presented with the focus on Ips typographus.

Keywords:*Bark Beetle aggregation, Non-Host Volatiles, repellent*

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Delivering robust semiochemical based crop protection via the seed

John Anthony Pickett
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Use of chemical ecological tools in crop protection is still considered by many practitioners, in both agriculture and the agricultural chemical industry, to be an inefficient option. However, pressure for sustainable crop protection without recourse to seasonal inputs reliant upon high carbon footprint, at least for delivery, requires us to look more diligently at approaches delivered by the seed. Breeding, even with new molecular approaches, and the first generation of genetically modified (GM) crops offer only limited solutions for pest control, still leaves synthetic chemical eradicant pesticides as the preferred option. Many of the latter compounds have been developed from natural product leads and, indeed, some are themselves natural and produced commercially by fermentor technologies. This means that the genetics associated with their biosynthesis can be elucidated further for exploitation, particularly for a new generation of GM crops. Most crops contain defence biosynthetic pathways which can, with recent developments in plant molecular genetics, be improved by novel breeding strategies, as well as by GM. By extending chemical ecological tools from metabolites acting directly on the physiological processes of pests, diseases and weeds, we can also consider targets that regulate development by acting as semiochemicals, including pheromones. Examples of all these approaches will be provided and discussed in the light of new opportunities from use of small lipophilic plant-derived compounds acting as elicitors of defence.

Keywords:*Genetic modification*

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Impacts of microbial associations on plant-insect

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PSU, United States Minor Outlying Islands

Plants inhabit complex environments in which they simultaneously interact with a diverse array of other species. Interactions with microbial pathogens and other symbionts can entail significant changes in plant chemistry that in turn affect interactions with insects, including disease vectors and non-vector herbivores. Recently we have been examining how a number of plant pathogens (e.g., viruses and bacteria) and mutualists (rhizobia) influence plant-insect interactions mediated by plant nutritional quality, defense chemistry,and volatile emissions. This work suggests that such effects can often be profound and in some cases can influence plant-insect interactions in ways that suit the interests of the microbial partners. For example, pathogen transmission mechanisms appear to be an important factor shaping the evolution of effects on host-vector interactions. I will present results from our most recent work and discuss implications for the chemical ecology of complex ecological communities.

Keywords:*plant-insect*

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S604W03

Behavior and Chemical Ecology

Airborne interactions between different plant cultivars- Effects on aphids and their natural enemies

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Plants damaged by herbivores or pathogens release volatiles active in defense signaling between or within plants, but undamaged plants may also engage in chemical interactions. We present results that contribute new perspectives on allelopathy and use of volatile cues by plants and insects. In a system consisting of different plant species and different barley genotypes we show that airborne signaling reduces attractiveness to an insect herbivore and affects interactions with the herbivore's predators. In barley, the inducing and responding capacity is shown to be genotype-related. Volatile profiling shows differences in the volatile blends released by different barley genotypes. Increased understanding of chemical interaction between undamaged plants may give a new outlook on the ecology of mixed genotype cropping and weed-crop interactions and their role in sustainable crop production.

Keywords:*allelopathy, aphids, natural enemies, semiochemicals*

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Attract to kill: enhancing effectiveness of mobile and immobile biological control agents

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Wageningen University , Netherlands

Biological control of arthropod pests is a durable method that is extensively used worldwide. Natural enemies of arthropod pests are used to keep the pest population below the economic injury level. Biological control agents include predators, parasitoids, viruses, bacteria and nematodes. Many effective natural enemies are able to find individuals of the pest species at low densities. This may involve the use of chemical cues originating from the pest or its food. Also arthropod pests exploit chemical cues to find their food including our crops and our animals. In order to enhance biological control we may exploit the involvement of chemical cues in the biology of pests and their natural enemies. We may devise methods to enhance the location of a pest by its natural enemy or to attract the pest to a site where we apply a natural enemy that does not actively locate its victim. Examples for predatory arthropods and for entomopathogenic fungi will be provided, including options to exploit chemical ecological knowledge in pest control.

Keywords:*chemical cues - arthropod behaviour - predators - entomopathogenic fungi - biological control*

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Exploiting chemical ecology of insect-plant and plant-plant interactions in developing novel IPM strategies for Africa

Zeyaur Khan[1], Charles Midega[2], Toby Bruce[3], Tony Hooper[4], John Pickett[5]

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Development of a novel push-pull approach for integrated pest and weed management is described. Appropriate plants were discovered that naturally emit signaling chemicals (semiochemicals) that influence insect-plant interactions. Plants highly attractive for egg laying by stemborer pests were selected and employed as trap crops (pull), to draw pests away from the main crop. Of these, Napier grass, Pennisetum purpureum (Schumach), despite its attractiveness, supported minimal survival of the pests' immature stages. Plants that repelled stemborer pests, notably molasses grass, Melinis minutiflora P. Beauv., and forage legumes in the genus Desmodium, were selected as intercrops (push), which also attracted natural enemies of the pests. Desmodium intercrops suppressed parasitic weed, Striga hermonthica (Del.) Benth., through an allelopathic mechanism. Desmodium root exudates contain novel flavonoid compounds, which stimulate suicidal germination of S. hermonthica seeds and dramatically inhibit its attachment to host roots. The companion crops provide valuable forage for farm animals while the leguminous intercrops also improve soil fertility and moisture retention. The system is appropriate as it is based on locally available plants, not expensive external inputs, and fits well with traditional mixed cropping systems in Africa. To date it has been adopted by more than 47,000 smallholder farmers in East Africa where maize yields have increased from ~1?t ha?1 to 3.5?t ha?1. Opportunities for semiochemical delivery by companion plants, including plant-plant signaling and early herbivory alert, are being explored for developing future smart IPM strategies for Africa.

Keywords:*Push-Pull*

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New frontiers for manipulation of beneficial insects in crop protection: From chemical to multimodal communication.

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[1]Embrapa - Brazil, Brazil, [2]Embrapa - Brazil, Brazil, [3]Embrapa - Brazil, Brazil

Achieving Food and Environmental security is recognized as one of the main directions of research in biological and agronomical sciences over the next few decades. New approaches to minimize the use of pesticides and improve the quality and productivity of food is crucial. Two tools have been proposed to minimize insecticide application on arable crops: (i) the use of semiochemicals from plants and insects and (ii) natural enemies. Soybean is one of the most important cultures in Brazil and stink bugs are the principal pests all around the country. They have been partially controlled by egg parasitoids (Platygastridae) through inundative release. However, this approach has limitations because it is laborious and costly. An alternative approach could be the use of semiochemicals to behavioural manipulation of natural enemies for conservative biological control strategies. Platygastridae wasps to locate their host use either herbivore-induced plant volatiles, herbivore-defensive compounds, sex pheromones and other host producer cues, vibratory signals for instance, to find their host. Another alternative is the manipulation of plant volatiles by breeding, metabolic engineering or exogenous application of naturally-occurring elicitors of plant defence to manage parasitoids. Notwithstanding, the dosage, time of application, and other aspects need to be improved before extensive use of this strategy in soybean crops. The integrated use of semiochemicals with other cues could help to develop more efficient tools for this behavioural manipulation. We intend to present results about the multimodal communication of Platygastridae egg parasitoid wasps, and discuss future steps required prior to application in agriculture.

Keywords:*Semiochemicals, Vibrational Communication, Stink bugs, Egg Parasitoids*

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Sex pheromones of mirid bugs: Old problems and new solutions

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Mirid bugs are important pests of many crops world-wide. They have been a rich source of new compounds for chemical ecologists, but establishment of behavioural roles for the compounds and application in management of pest species have been less rewarding. Most mirid bugs have metathoracic glands producing compounds which may function as sex pheromones, alarm pheromones and defence compounds, greatly complicating their study and application. Bugs of the sub-family Bryocorinae are unusual in not having metathoracic glands. Two species, Distantiella theobromae and Sahlbergella singularis are pests of cocoa in West Africa and share two female-produced sex pheromone components. The synthetic compounds attract males when released from standard dispensers and use of pheromone traps for monitoring and control is being investigated. Bugs of Lygus and related genera are more typical with large metathoracic glands. Over the last 30 years, several species have been shown to produce three main compounds, hexyl butyrate, (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal, but their roles as components of sex pheromones have not been confirmed, at least in part because of the difficulty of dispensing these compounds in a specific blend over a significant period. We have studied four Lygus species and shown they produce these three compounds in characteristic ratios. Novel dispensers have been developed which release defined blends of the three components for at least one month under field conditions. When combined with suitable trap designs, good attraction of males has been demonstrated, opening the way to new approaches for management of these pests.

Keywords:*mirids, pheromones*

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A first step towards the control of a major root pests: Using chemical ecology to understand why it is a pest

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University of Neuchatel, Switzerland

We use a chemical ecological approach to better understand the interactions among maize plants, pest insects and the natural enemies of the pests. These efforts have led to an explanation for the voraciousness of one of the most important pests of maize, the Western corn rootworm or Diabrotoca virgifera virgifera. The larvae of this beetle cost American farmers an estimated two billion dollars annually. Since its incidental introduction into the Balkan region in the 1990s it has rapidly spread and become a serious problem in Europe as well. A series of chemical analytical experiments and feeding assays that included mutant maize plants we think we have revealed the reason why the insect is so successful on maize. The larvae were found to prefer feeding on nutritious crown roots of maize plants. These roots are essential for optimal performance of the plant and logically well-defended, mainly by toxic benzoxazinoids that normally deter herbivores from feeding on these roots. The rootworm larvae, however, are completely unaffected by the toxins and use them to identify the nutritious roots, which are rich in sugars and amino acids. This explanation for the success of D. virgifera on maize opens the way for new approaches to control this pest. We argue that in general, a good understanding of chemically mediated interactions among plants and insects is essential for the development of novel and ecologically sound strategies for crop protection.

Keywords:*Root pests, maize, induced defence, induced susceptibility, Diabrotica, rootworm*

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Elucidating the chemical ecology of belowground plant-plant communication

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In pioneering studies on plant-aphid interactions, we have observed that when bean plants, Vicia faba, are infested with pea aphids, Acyrthosiphon pisum, they transmit signals via the rhizosphere that induce aboveground defence in intact, neighbouring plants. In wind tunnel assays, the generalist aphid parasitoid, Aphidius ervi, is significantly attracted to intact V. faba grown in hydroponic solution previously used to grow A. pisum-infested plants. To identify the rhizosphere signal(s) which mediate belowground communication, root exudates were collected from hydroponically-grown 10 day-old A. pisum-infested and uninfested V. faba plants, and exudates subjected to reverse-phase (C18) solid-phase extraction (SPE). When SPE extracts were re-constituted in hydroponic water subsequently used to grow intact V. faba, the extract from A. pisum-infested V. faba caused intact plants to become attractive to A. ervi. Preliminary results demonstrate a role for small lipophilic molecule (SLM) chemical signals in belowground plant-plant communication following aphid colonisation. The isolation and identification of the biologically active chemical(s) via bioassay-guided HPLC fractionation is currently under way.

Keywords:*Aphidius ervi, Acyrthosiphon pisum, Vicia faba, hydroponic water, root exudates*

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Elicitation of plant defence by small lipophilic molecule activators: Understanding underlying mechanisms and towards use in crop protection

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Small lipophilic molecule (SLM) activators of plant defence offer a route to sustainable crop protection, by inducing plant secondary metabolism pathways that provide either direct or indirect defence against insect pests. Although they can potentially be deployed directly as crop protection agents, the long term vision is to use activators as novel tools in the selection, and breeding, of resistant traits into new varieties and cultivars that respond rapidly to insect damage. It is envisaged that this strategy will deliver sustainable crop protection via the seed. Seminal studies on the interaction of aphids and their natural enemies, parasitoids and predators, with host plants showed that the interaction is modified by the activation of defence signalling by cis-jasmone, a volatile plant secondary metabolite. The mechanisms underlying activation of defence by cis-jasmone were studied in the model plant Arabidopsis thaliana and in wheat, Triticum aestivum, and the potential for using cis-jasmone as a practical tool for activation of defence in major world crops was explored. Stress signalling is activated in cotton, Gossypium hirsutum, and soybean, Glycine max, and is primed in maize, Zea mays. These studies demonstrate promise for the use of SLMs as natural plant activators in the sustainable control of pest aphids, stink bugs and leafhoppers. To this end, new classes of SLM activators are being investigated, including those associated with below-ground plant/plant communication in field beans, Vicia faba, following above-ground aphid herbivory, and with induction of indirect defence in maize following herbivore oviposition.

Keywords:*Plant defence, elicitation, cis-jasmone, crop protection, SLMs*

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Patterns of attraction of herbivore pests to plant volatiles

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Plant volatiles play an important role in the host and mate finding behaviors of insect herbivores, and understanding the mechanisms of this process can provide tools for pest management. Here we used a meta-analysis to synthesize this knowledge and to look for patterns on the effects of plant volatiles on insect pests. Our goal was to identify herbivore and plant traits that might explain the herbivores' behavioral response to plant volatiles in field applications. We used a total of 374 unique plant volatile-insect herbivore interactions obtained from 34 published studies investigating 50 herbivore pest species in our analysis. We found that attractants, but not repellents, had a significant effect on insect herbivore abundance; this difference could be due to the smaller number of field studies that tested plant volatiles as repellents (3%). Females were significantly more attracted to plant volatiles than males. The diet breadth of herbivores was independent of a behavioral response to plant volatiles, but the effect of volatiles was the greatest on chewers, followed by wood-borers and sap-feeders. There are more demonstrations of attraction to plant volatiles in Lepidoptera than in Thysanoptera. The method of plant volatile application had a significant effect on herbivore abundance and increasing the number of chemicals in blends attracted more herbivores. The magnitude of the response of herbivores to plant volatiles in forest and agricultural habitats was similar. These data were published in 2010 (Ent. Exp. Appl. 134:201). We will highlight areas needing research in using plant volatiles to manage insect pests.

Keywords:*plant volatiles, host finding, pest management*

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S605TH02

Behavior and Chemical Ecology

Influence of mating on responses of *Helicoverpa armigera* moths to synthetic plant volatiles

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Responses of mated and unmated Helicoverpa armigera female moths to various synthetic plant volatile compounds were studied using a two-choice olfactometer. Results suggest that mated moths are both more active in the olfactometer and more responsive to most compounds than unmated ones. Even in a blank olfactometer (ie, no volatile) or where only the carrier canola oil was present, there was a trend for more mated than unmated moths in the test chamber, although the differences were not statistically significant. There was also a trend for the difference between mated and unmated moths to be greater with more attractive compounds. We also compared mated females with females which had been confined with another female for an equivalent period to that which the mated females spent with males, to determine whether it was mating per se that influenced responsiveness in the olfactometer, or just confinement with another moth. There was no difference between these two groups of females, suggesting it is mating which is the crucial factor.

Keywords:*Helicoverpa, plant volatiles, olfactometer, mating*

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S605TH03

Behavior and Chemical Ecology

How fruit moths recognize host plant odors

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Chemically-mediated host plant selection by female herbivores may seem to be a straight-forward interaction between plants and females searching for suitable oviposition sites. However, recent advances in research on plant-herbivore relationships reveal a more complex situation with important consequences. Using the model system composed of the invasive fruit moth Grapholita (=Cydia) molesta and volatiles from its primary host peach, we addressed some key aspects of the females' olfactory response to host plant volatiles. Starting from the complex volatile blend emitted by the plant, we simplified the mixture in a stepwise manner while maintaining its attractive properties. With this top-down approach, we detected a synergistic interaction between two constituents, which are behaviorally ineffective when tested alone. Neurophysiological work confirmed this synergism conclusively. Motivated by the observation that single females respond differently to the same odor blend, we investigated the heritability of odor-guided host location behavior in this species using a full-sib/half-sib approach. Results documented a considerable genetic basis for olfactory attraction of females. Moreover, there is a genetic trade-off between female attraction and an important life history trait. This trade-off might be attributable to conflicting resource allocation, and it highlights the inherent constraint for simultaneous selection for both traits. Invited submission: Chemically-mediated host plant selection, a complex and challenging topic (6-4.)

Keywords:*Grapholita molesta, volatiles, behavior, trade-off, heritability*

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Microbial odors mediate host finding in insect herbivores

Peter Witzgall
SLU, Sweden

Attraction of the fruit fly D. melanogaster to a blend of yeast volatiles with acetic acid as a main attractant compound (Becher et al. 2010, 2011) demonstrates the significance of yeasts in Drosophila behavioral ecology. With this in mind, we have investigated the role of yeasts for host finding and oviposition in other insect herbivores. We conclude that plant-yeast-insect interactions are more widespread than previously assumed, and that yeasts and other micro-organisms are important for our understanding of the ecology of insect herbivores and their evolutionary diversification. The traditional bi-trophic plant-insect niche concept must be updatedto accomodate for the role of micro-organisms in host-finding. This will be illustrated with results from current studies. Becher PG, Bengtsson M, Hansson BS, Witzgall P. 2010. Flying the fly: long-range flight behavior of Drosophila melanogaster to attractive odors. J chem Ecol 36:599-607 Becher PG, Flick G, Rozpedowska E, Lebreton S, Larsson MC, Hansson BS, Piskur J, Witzgall P, Bengtsson M. 2011. Yeast links the fly to fruit. (submitted)

Keywords:*insect, herbivore, yeast, host finding, plant, interaction, semiochemicals*

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Attacking behavior and its communication of an invasive bark beetle *Dendroctonus valens*

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[1]*Institute of Zoology, Chinese Academy of Sciences, China,* [2]*Institute of Zoology, Chinese Academy of Sciences, China*

The invasive red turpentine beetle, Dendroctonus valens, is one of the most destructive forest pests in China, which is a secondary pest in its native range North America. It is urgent to understand its special behavior and underlying communications since the bark beetle mainly attack Chinese red pine Pinus tabuliformis, which is the main plantation in North China. Field investigation showed that RTB prefers to attack large pine trees and further analysis showed it is mediated by monoterpenes variation among variable size of pine trees. In field, RTB female adult initiates attack first, and one female and one male are in the same tunnel normally, which is the evident of monogamy. How this family structure is maintained? It shows that male who joined with female already, can prevent other male to meeting with its partner through the sound of stridulation to keep other males away. Besides, volatiles bioassay shows some pheromones have involved and GC-MS analysis shows that frontalin is released by feeding females, which functions as both sex and aggregation pheromone. Brevicomin is produced by male and acted as anti-aggregation pheromone, playing an important role in host shifting. Furthermore, male mating selection is evident that males always find large females to mate with, which could have more offspring. Underlying mechanisms are explored and shows that male could discriminate the size of tunnel and female itself through the amplitude (dB) of the drum-like sound produced by female. These are very useful to understand the invasion biology and to guide management.

Keywords:*Bark beetle Dendroctonus valens, attack behavior, chemical communcations, acoustic signal.*

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S605TH06

Behavior and Chemical Ecology

Do novel fungal genotypes drive the success of an invasive bark beetle/pine host/fungus complex?

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Novel genotypes often arise during biological invasions, but their role in invasion success has rarely been elucidated. Here we examined the population genetics and behavior of the fungus, Leptographium procerum, vectored by a highly invasive bark beetle, Dendroctonus valens, to determine whether genetic changes in the fungus contributed to the invasive success of the beetle/fungal complex in China. The fungus was introduced by the beetle from the US to China, where we identified several novel genotypes using microsatellite markers. These novel genotypes were more pathogenic to Chinese host seedlings than were other genotypes and they also induced the release of higher amounts of 3-carene, the primary host attractant for the beetle vector, from inoculated seedlings. This evidence suggests a possible mechanism, based on the evolution of a novel genotype during the two or three decades since its introduction, for the success of the beetle/fungal complex in its introduced region.

Keywords:*invasion mechanism, chemical ecology, fungal genetics, bark beetles-ophiostomatoid fungi-hosts inter*

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The role of kairomones in the mating sequence of cerambycid beetles

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Regardless of whether or not species utilize pheromones, host kairomones play a critical role in the chemical ecology of the Cerambycidae, and are perhaps the most critically important, yet least understood steps in the sequence of reproductive behaviors of cerambycid beetles. Patterns are beginning to emerge regarding the various roles that host kairomones play in the lives of cerambycid beetles, and using recent empirical evidence, a synthesis of these ideas will be presented. Considerable research effort has been invested in developing pheromone-based lures for invasive or potentially invasive species, and there has been a recent surge in the number of species for which pheromones have been discovered, however, the exact roles of kairomones remains elusive. For some Monochamus and Tetropium spp., attraction to pheromones are synergized with host kairomones, while for other species, such as Anoplophora glabripennis, the exact role of host kairomones is not apparent. Previous reviews (Hanks 1999) have addressed how mate location strategies may be predicted by host condition. In this talk, the hypothesized mate finding 'strategies' of two pheromone-using cerambycids, one attacking stressed hosts, and the other attacking apparently healthy hosts, will be compared by discussing how host kairomones relate in the first step of the mating sequence in each 'strategy' (host selection), and then discuss how host kairomones are involved with female-produced and male-produced pheromones later in the sequence. Reference: Hanks 1999. Ann. Rev. Entomol.

Keywords:*host kairomones, pheromones, Cerambycidae, mating sequence*

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Chemo-ecological risk assessment of European beech (*Fagus sylvatica*) stands along a precipitation gradient in Germany

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Volatile organic compounds (VOC), insect biodiversity and the olfactory perception of marker insect species were examined in European beech stands along a precipitation gradient in Northern Germany. Firstly, drought specific VOC as released by beech trunks and as detectable in beech stand air were measured throughout the years 2010/2011. In parallel, the species assemblies of phyllophagous insects, of xylophagous insects, and of antagonists were monitored. Secondly, beech specific insects were selected to examine their ability to detect drought specific VOC of beech. Olfactory detection of beech VOC was assessed utilizing gas-chromatography with parallel mass-spectrometric and electroantennographic detection (GC-MS/EAD). The identification of VOC specifically released by weakened beech trees and detected by insects attacking beech stands is an important step towards a chemo-ecologically based risk assessment of insect gradation. Utilizing biosensors on the basis of intact insect antennae can be a mean to develop a highly specific early warning system for forest stands under stress and prone to accelerated decline by secondary insect pests.

Keywords:*beech, VOC, drought, xylophagous insect, phyllophagous insect, electrophysiology*

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Direct and indirect effects of a Tospovirus on the behavior and biology of *Frankliniella fusca*, a Tospovirus vector

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Several circulative viruses are known to modify the behavior and biology of their vectors to benefit their own survival and spread. Tospoviruses are transmitted by thrips in a circulative and propagative manner. Very few studies conducted with a Tospovirus, Tomato spotted wilt virus (TSWV), and its vector, Frankliniella occidentalis (Pergande), have yielded mixed outcomes. Using peanut plants, we evaluated the effect of TSWV infection on settling and feeding behavior of Frankliniella fusca (Hinds). Also, effects on thrips oviposition and development were evaluated. TSWV infection in thrips and/ or peanut plants did not affect their settling pattern, but TSWV infection in thrips and peanut significantly impaired thrips feeding ability. TSWV infection in thrips increased their egg-laying capacity, but TSWV infection in peanut plants decreased their oviposition ability. In general, infections by circulative viruses are known to increase the concentrations of free amino acids in host plants, and amino acids have been known to influence oviposition in several insects. Evaluation of free amino acids in TSWV-infected and non-infected peanut plants indicated the presence of greater amounts of free amino acids, suggesting that amino acid increments could have led to increased thrips oviposition. TSWV infection in thrips significantly affected their survival and development. Despite the spike in free amino acid levels, TSWV-infection in peanut, overall, negatively affected the reproductive fitness of F. fusca. This suggested that free amino acids and possibly others might play a role in deceptive arrestment of thrips on TSWV-infected plants. Such an arrestment might be beneficial for TSWV spread.

Keywords:*Thrips, Tospovirus, Interactions*

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S605TH10

Behavior and Chemical Ecology

How flowers attract pollinator beetles in annonaceous fruit trees?

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The tropical fruit trees, cherimoya (Annona cherimola) and atemoya (A. cherimola x squamosa) are pollinated mainly by sap beetles (Coleoptera: Nitidulidae), which are attracted to floral odor. However, usually the natural fruit set is not enough so that hand pollination is required. To understand this rather primitive pollination system to replace costly hand pollination with beetle pollination, we explored chemical component of the floral odor of these trees. Odor was collected by in situ dynamic head space technique, and analyzed by GC and GC/MS. Most of the components were esters, with ethyl hexanoate, ethyl butanoate, ethyl octanoate, and ethyl hexanoate being main components. Protogynous hermaphrodite flower of these trees exhibit somewhat different composition of these esters between species and sexual stages (we could not obtain enough data for male stage flower of atemoya). As the result, principal component analysis clearly separated the two species and sexes. Because esters are main components of fruit odor in general, we hypothesized that theese flowers mimic fruits chemically to attract frugivorous sap beetles. We thus examined literature for data of fruit odor of cherimoya, atemoya and other fruit trees, as well as floral odor of other trees. We then employed principal component analysis for these data. The result showed that cherimoya and atemoya flowers are chemically very similar to their own fruits and fruits of other trees. We also examined quantitative change of the floral odor. The result will be discussed with diurnal rhythm of the pollinator beetles.

Keywords:*beetle pollination, Annonaceae, Nitidulidae, flower odor, GC, MS, cherimoya, atemoya*

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Odorant activation of insect olfactory receptors is inhibited by antagonism of the odorant receptor co-receptor subunit

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Insects detect attractive and aversive chemicals using several families of chemosensory receptors, including the OR family of insect odorant receptors, making these receptors appealing targets for the control of insects involved in disease propagation and agricultural damage. Insect ORs are odorant-gated ion channels, consisting of two essential parts: an odorant binding subunit and a co-receptor subunit (Orco). VUAA1 (CAS: 525582-84-7) was recently identified as a novel OR agonist that acts directly on Orco (Jones et al., 2011, PNAS 108: 8821-5). We screened ORs from several insect species using heterologous expression in Xenopus oocytes and electrophysiological recording, with a panel of compounds (termed Orco Ligand Candidates, OLCs) that were structurally related to VUAA1 (4-Ethyl-1,2,4-triazolothioacetamide core with alterations to the pyridine and phenyl rings) or similar to portions of the VUAA1 structure. OLC3 (pyridine nitrogen moved to the 4 position) and OLC12 (pyridine nitrogen in the 4 position and a 4-isopropyl moiety on the phenyl ring) were identified as agonists of the Orco subunit. We also identified a series of antagonists, each able to competitively inhibit OLC12 activation of Orco. A similar pattern of agonist and antagonist sensitivity was displayed by Orco subunits from different species, suggesting a highly conserved binding site structure. Importantly, we found that Orco antagonists could inhibit odorant activation of insect ORs through a non-competitive mechanism. Susceptibility to inhibition of odorant activation by Orco antagonism was conserved across disparate insect species, identifying Orco antagonism as a promising target for the development of novel, broadly active insect repellants.

Keywords:*olfactory receptor, Orco, allosteric antagonism, electrophysiology*

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S606TH03

Behavior and Chemical Ecology

Evolution of sex pheromone production and reception systems in Ostrinia moths

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Mate-finding communication in many moths is mediated by the sex pheromones produced by females. Since differentiation of sex pheromones is often associated with speciation, it is intriguing to know how the changes in female sex pheromone have been tracked by the pheromone recognition system of the males. Moths of the genus Ostrinia, which show distinct differentiation in female sex pheromones, are good materials for studying the evolution of pheromone communication system. To detect the sex pheromones emitted by the conspecific females, males should have a system that can discriminate pheromone components and their ratios. In this system, odorant receptors (ORs) in the olfactory sensilla of male moths should play an important role in the specific recognition of pheromone molecules. We cloned seven OR genes (OscaOR1, OscaOR3?8) and an Orco homologue (OscaOR2), which is essential for the function of ORs, from the antennae of male Ostrinia moths. The results of functional assay of these ORs to pheromone components utilized in the genus Ostrinia will be reported. The response patterns will be discussed in terms of the evolution of sex pheromone production system in this genus.

Keywords:*Ostrinia, Odorant receptor, Orco, Sex pheromone*

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Peripheral coding of sex pheromone blends with reverse ratios in two Helicoverpa species

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The two sibling sympatric species, Helicoverpa armigera and Helicoverpa assulta share two major sex pheromone components, cis-11-Hexadecenal and cis-9-Hexadecenal, but in reversed relative concentrations, 97:3 and 7:93, respectively. We combined behavior, electrophysiology, molecular biology and optical imaging to investigate the peripheral coding mechanisms in males to their sex pheromone blends with reverse ratios. Two groups of highly specific olfactory receptor neurons (ORNs) in two types of antennal sensilla are mainly involved in the pheromone coding, and each of them are tuned to one of the two components. As labeled lines, the two groups of ORNs each send unique information based on their firing rate, and continuously signal to the antennal lobe the levels of cis-11-Hexadecenal and cis-9-Hexadecenal in the air. The different sensitivity, abundance and distribution of the ORNs of each type further increase the differences between the two signals, detecting the ratio and reflecting importance of two components in the pheromone system of the two species. The parallel studies on the hybrids (F1) between H. armigera and H. assulta indicate that the number of ORNs of each type is an important factor affecting the accuracy of ratio detection. The olfactory receptors and associated transduction molecules contribute to the specificity and sensitivity of ORNs to the signals. The projection of the axons of ORNs to the male specific macroglomerular complex in the antennal lobe was also different between the two species. The central nervous system may compare the paired input and accordingly trigger the appropriate behavior.

Keywords:*Sex pheromone, peripheral coding, olfactory receptor neuron, olfactory receptor, Helicoverpa*

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S606TH05

Behavior and Chemical Ecology

A peripheral 'memory' in the *Drosophila* olfactory system

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Insects are heavily dependent on a well functioning olfactory system for more or less all activities. A plasticity in this system has also been shown to be advantageous. Here I will report on new data regarding the function of the insect olfactory co-receptor Orco. We have found that in the fruit fly Drosophila melanogaster Orco might work as a regulator of peripheral sensitivity . The data is unpublished but under review. I therefore provide no details. Please visit my talk.

Keywords:*Orco, Drosophila, olfaction, transduction*

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Molecular genetic studies on the spli locus controlling pheromone preference in the silkmoth, *Bombyx mori*

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We have started the molecular genetic analysis of the 'polyphagous' mutants of B. mori, which feed not only mulberry but also the beet and other plant leaves. We succeeded in the positional cloning of two mutants, spli and Bt mapped on the Z chromosome, and found that both are alellic mutations of Bmacj6, a gene encoding a transcription factor with the POU-homeo domain. Recently, we found that the spli and Bt mutations do not affect not only food preference but also pheromone preference. Although the normal male adult is attracted by the female sex pheromone 'bombykol' ((10E,12Z)-hexadecadienol), the spli male is attracted by the aldehyde derivative, 'bombykal' ((10E,12Z)-hexadecadienal) (1). These results indicate that Bmacj6 controls both the food preference and pheromone preference through unknown transcriptional regulations. The mRNA expression of the bombykol receptor gene (BmOr1) was dramatically reduced in the adult male antennae of the mutants. We will introduce the recent progress in the comparative transcriptome analysis for finding the direct and indirect target genes of BmAcj6.
Reference: Fujii, T., Fujii, T., Namiki, S., Abe, H., Sakurai, T., Ohnuma, A., Kanzaki, R., Katsuma, S., Ishikawa, Y., and Shimada, T. (2011) Sex-linked transcription factor involved in a shift of sex pheromone preference in the silkmoth, Bombyx mori. Proc. Natl. Acad. Sci. USA. 108: 18038-18043.

Keywords:*behavioral mutant, positional cloning, sex-linked gene, odorant receptors*

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Coevolution of moth pheromone production and response in two model systems

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Communication depends on the mutual understanding of senders and responders. Sex pheromone communication in moths involves production of species-specific pheromones by females and their detection by conspecific males. Genes involved in pheromone production and reception have emerged via a birth-and-death evolutionary model. Enzymes and receptors with different specificities and sensitivities have evolved as a consequence of gene duplications and the resulting alteration of selective pressure and accumulation of mutations in daughter gene copies. We study two model systems from the perspective of pheromone evolution, the European corn borer Ostrinia nubilalis and the turnip moth Agrotis segetum. Pheromone production genes in O. nubilalis map to autosomes whereas three receptor genes with fixed allelic differences between pheromone races map to the male sex chromosome. Males and females produce similar pheromones by enzymes common to both sexes. The pleiotropic effects of genes could influence the trajectories of sexual signals and facilitate their divergence. Agrotis segetum produces a mixture of pheromone components including Z5-10:OAc, Z5-12:OAc, Z7-12:OAc and Z9-14:OAc. These and the behavioural antagonist Z5-10:OH and Z8-12:OAc elicit responses from specific receptor cells on the male antenna. Nine candidate pheromone-receptor (PR) genes belong to a subclade expansion previously described in Heliothis virescens PRs. Six of them cluster in the expansion of HR16 and show high amino acid sequence identity (71.0-97.7%). This suggests rapid evolution of PR genes in A. segetum. Functional characterization using Xenopus oocytes shows that substitutions at a few sites account for changes in ligand specificity and sensitivity.

Keywords:*receptor, OR, PR, evolution*

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Working Range of Stimulus Flux Transduction Determines Dendrite Size and Relative Number of Pheromone Component Receptor Neurons in Moths

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We are proposing that the relative abundances of the differently tuned pheromone-component-responsive olfactory receptor neurons (ORNs) on insect antennae are a result of specifically tuned sensillum-plus-ORN units having been selected to accurately transduce and report to the antennal lobe the maximal ranges of molecular flux imparted by each pheromone component in every plume strand. To not reach saturating stimulus flux levels from the most concentrated plume-strands of a pheromone blend, the dendritic surface area of the ORN type that is tuned to the most abundant component of a pheromone blend is increased in dendritic diameter in order to express a greater number of major-pheromone-component-specific odorant receptors (ORs). However, with the larger dendrite size and possibly other, high-flux-adjustments in titers of pheromone binding proteins and degrading enzymes comes a decrease in absolute sensitivity to lower flux levels of the major component in lower concentration strands of the pheromone blend. In order to restore the ability of the whole-antenna major-pheromone-component-specific channel to accurately report to its glomerulus the abundance of the major component in lower concentration strands, the number of major-component ORNs over the entire antenna is adjusted upward, creating a greater proportion of major-component-tuned ORNs than those tuned to minor components. Thus, we suggest that the two phenomena, dendrite size and relative numbers of differentially tuned ORNs are linked, and both are related to wide disparities in molecular flux ranges occurring for the more abundant and less abundant components in the pheromone blend plume-strands.

Keywords:*Odorant flux*

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S607F01

Behavior and Chemical Ecology

A novel control strategy against an invasive Argentine ant population by combining an insecticidal bait with a synthetic trail pheromone component

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Control of the invasive Argentine ant, Linepithema humile in invaded areas is highly difficult. A major reason is undoubtedly formation of large-scale supercolonies (SCs). For effective control, treatment should be area-wide according to SC size, but it is virtually impossible to treat toxic agents throughout the habitat. Local treatment within a SC would cause rapid re-infestation in the treated area from surrounding untreated area. We then tried to incorporate synthetic trail pheromone (Z)-9-hexadecenal as a non-toxic as well as species-specific control agent into a control program. Based on the positive results obtained in preliminary small-scale field experiments with both insecticidal baits and a synthetic trail pheromone, we achieved a program to eradicate an Argentine ant population in Yokohama Port, one of major ports of entry, Japan. The population was first found at the port in 2007 and covered an area of about 6.7 ha. We initiated the program in April, 2008; insecticidal baits were deployed to suppress the population, and pheromone dispensers were treated to prevent expansion of its range. Within 18 months of the program, L. humile was eliminated from most of the study area using a relatively small amount of insecticidal bait. Expansion of its range did not occur during program, whereas native ants showed a marked recovery in abundance in the area once dominated by L. humile. The achievement at Yokohama Port may be a good example of controlling invasive ants at ports of entry, providing useful suggestions for effective and environmentally friendly control.

Keywords:*invasive ants, Linepithema humile, eradication, synthetic trail pheromone, insecticidal bait*

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S607F02

Behavior and Chemical Ecology

Control of *Riptortus pedestris*: Pheromone synthesis and trap development

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Sweet (non-astringent) persimmon, Diospyros kaki Thunberg, is one of the important export fruits of Korea. For development of monitoring techniques and environmentally-friendly control strategies against the pests for exporting the fruit, integrated pest management is under investigation. Riptortus pedestris F. (= R. clavatus) (Hemiptera: Alydidae) is one of problematic pests of sweet persimmon in Korea and soybean in Asian countries. Monitoring and mass trapping of R. pedestris based on its aggregation pheromone has been applied by some famers. For enhancing pheromone-based control against R. pedestris, we developed new synthetic method for (E)-2-hexenyl (E)-2-hexenoate (E2HE2H) and (E)-2-hexenyl (Z)-3-hexenoate (E2HZ3), two components of the aggregation pheromone of R. pedestris, and also a new type of pheromone-baited trap. E2HE2H and E2HZ3H were synthesized by applying Steglich esterification method in various conditions. This synthetic method gave satisfied yield, shortened the synthetic process and lowered the cost. The newly designed pheromone trap, named symmetry trap, showed 6-28 times more trapping efficiency than a conventional trap. In this symposium, the results of synthesis for E2HE2H and E2HZ3H and the trap efficiency of newly developed trap will be discussed.

Keywords:*Riptortus pedestris (=P. clavatus), (E)-2-hexenyl (E)-2-hexenoate, (E)-2-hexenyl (Z)-3-hexenoate, symmetry trap*

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S607F03

Behavior and Chemical Ecology

Success and fail in monitoring two sibling species of genus Matsumuraeses (Tortricidae) with same sex pheromone components in a same field

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Matsumuraeses phaseoli and M. falcana are main insect pests to legumes. Laboratory colonies were established from larvae collected in red bean for M. phaseoli and in soybean for M. falcana, respectively in different regions. Sex pheromone studies were conducted to make monitoring tools for two species. The sex attractant of M. falcana was decided only through field experiments, while that of M. phaseoli was decided through analyses of pheromone gland extract and field experiments. It was concluded that the same three compounds, (E)-8-dodecenyl acetate, (E,E)-8,10-dodecadienyl acetate and (E,Z)-7,9-dodecadienyl acetate are used for the sex attractants of two species, but in different compositions, 1:7:2 for M. phaseoli, and 1:1:1 for M. falcana. The attractants were used for monitoring of annual occurrence of Matsumuraeses adults in a same field. The morphology of male genitalia was used for species-identification of adults caught in pheromone traps. Both species, however, were caught in the pheromone trap of each species. A clearly-different character between the two species was found in a nucleotide sequence of cytochrome oxidase I gene. The identification with the molecular character also showed the same results as the catch of both species in the pheromone trap of one species. Virgin female traps also gave the same results as the pheromone traps. The results indicated that the occurrence patterns of two species observed with pheromone trap should be corrected. Artificial hybridization experiments were conducted to confirm the sexual isolation between the two species. Finally, developmental characters were compared to analyze the monitoring results.

Keywords:*Matsumuraeses phaseoli, M. falcana, sex pheromone, monitoring, co-attraction*

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S607F04

Behavior and Chemical Ecology

Sex pheromone blends for monitoring clearwing moths in Korean orchards

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Clearwing moths (Lepidoptera: Sesiidae) are economically important pests of fruit trees, such as apple, pear, peach, and grape in Korea. Larvae feed on the cambial issue within tree branches of host plants, resulting in significant production losses. In this study, sex pheromone system and seasonal flight activity of four clearwing moths, Synanthedon haitangvora, Synanthedon bicingulata, Nokona regalis, and Glossosphecia romanovi, were studied. In field tests conducted at apple orchards, male S. haitangvora were optimally attracted to traps baited with a 5:5 mixture of Z3,Z13-18:OAc and E2,Z13-18:OAc. The maximum number of males S. bicingulata was attracted to a 4:6 blend of E3,Z13-18:OAc and Z3,Z13-18:OAc in peach orchards. While E3,Z13-18:OH as a single component was attractive to male N. regalis, high numbers of G. romanovi males were captured with a 9:1 ratio of Z3,Z13-18:OH and Z3,Z13-18:OAc in vineyards. The seasonal flight patterns of four clearwing moths were monitored with pheromone traps in Korean orchards. Male S. haitangvora were captured from May to October, with peak numbers in early June to middle June and late August to early September in apple orchards. S. bicingulata males were trapped from May to October, with peak catches in middle May to late May and early September in peach orchards. In vineyards, male N. regalis were captured from late May to middle June, but G. romanovi males were trapped from early June to late July.

Keywords:*Lepidoptera, Sesiidae, Sex pheromone, monitoring, fruit tree*

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S607F05

Behavior and Chemical Ecology

Sex pheromone composition of *Ascotis selenaria* (Lepidoptera: Geometridae) and its regional variation in Korea

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This study was conducted to investigate sex pheromone composition of Ascotis selenaria (Lepidoptera: Geometridae) in Korea. Two sex pheromone compounds such as (Z,Z)-6,9-cis-3,4-epoxynonadecadiene (6Z,9Z-cis-3,4-epoxy-19:H) and (Z,Z,Z)-3,6,9-nonadecatriene (3Z,6Z,9Z-19:H) were identified in the glands of A. selenaria females by gas chromatography?mass spectrometry analysis. However, the component 3Z,6Z,9Z-19:H neither elicited an electroantennogram response nor increased the attractiveness for A. selenaria males in the field. The role of 3Z,6Z,9Z-19:Hseems to be as an antagonistic signal formating behavior of A. selenaria males. The blend ratios of two 6Z,9Z-cis-3,4-epoxy-19:H isomers such as, 6Z,9Z-cis-3R,4S-epoxy-19:H and 6Z,9Z-cis-3S,4R-epoxy-19:H, were critical to attract A. selenaria males. The blend ratios of the two isomers showing peak catch of A. selenaria males had large variations among the locations investigated. A. selenaria populations in Gunwi showed peak activity at ratios of 0.9:0.1 and 0.8:0.2, whereas the populations in Goheung, Yeongam, and Jeju (Aewol and Harye) showed peak activity at a 0.5:0.5 ratio. In Changnyeong, the peak activity occurred in a bimodal form at ratios of 0.7:0.3 and 0.4:0.6. Such variation was partially explained by geographical isolation due to mountain ranges. Consequently, the results of our study should be useful for designing a region-specific pheromone lure for successful A. selenaria monitoring.

Keywords:*Sex pheromone composition of A. selenaria in Korea*

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S607F06

Behavior and Chemical Ecology

Fire ant alarm pheromone and application

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The red imported fire ant, Solenopsis invicta, is among the world's 100 worst invasive alien species. In the United States this ant species infests more than 130 million hectares in southern tier states and Puerto Rico and are spreading northward. It is estimated to be responsible for almost $7 billion annually in damage repair, medical care, and control costs. The affected economic sectors are broad ranging and include households, electric service and communications, agriculture, schools and recreation areas. In the last decade S. invicta has changed from an invasive pest ant in the United States to a global problem, with infestations occurring in Australia, Taiwan, mainland China, Mexico and many Caribbean Island countries. Thus, novel control methods are needed and could result from manipulation of the fire ant alarm pheromone to disrupt mating flights or to in some way disrupt alarm pheromone production that could interrupt the normal insularity of fire ant colonies. We explored the changes of the amount of alarm pheromone amount in workers, female and male alates, and queens. We discovered that the pattern of alarm pheromone accumulation is different in the above distinct female castes and mating status. These results presented here could be lead to an understanding of how the alarm pheromone is used by fire ants in the various contexts of workers, female alates, and queens. This is the initial step in developing a novel fire ant control method from the interference of alarm pheromones in colony defense, mating flights, and colony development.

Keywords:*ant, pheromone, alarm, neuropeptide*

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S607F07

Behavior and Chemical Ecology

Effect of minor components on mating disruption of light brown apple moth

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A new four-component sex pheromone blend for light brown apple moth (Epiphyas postvittana) was tested for efficacy of mating disruption in laboratory and field trials. Vineyard trials examined: a) the current two component ((E)-11-tetradecenyl acetate and (E,E)-9,11-tetradecadien-1-yl acetate, 95:5), b) a new four-component blend ((E)-11-tetradecenyl acetate and E,E-9,11-tetradecadien-1-yl acetate plus (E)-11-tetradecen-1-ol and (E)-11-hexadecenyl acetate (10:0.5:0.1:0.05)), c) higher ratio of the two new minor compounds (10:0.5:10:10), d) extra minors with an antagonist ((Z)-11-tetradecenyl acetate) added, e) extra minors with antagonist minus the most expensive component (E,E-9,11-tetradecadien-1-yl acetate), compared to f) untreated controls. In the laboratory, 69.0% of untreated pairs mated, and mating was suppressed by both attractive blends (41.1% mating (P=0.024, two component blend), and 18.4 % mating (P=0.003, four-component blend). In vineyards, totals of 24,776 and 41,053 male moths were caught in two trials, respectively, in delta traps baited with four-component lures and virgin females. Mating disruption was achieved at a higher level with a four-component blend for 13 / 14 weeks in trial 1 and for 10 / 10 weeks in trial 2. Disruption was improved by 12% with the four-component blend compared to the two-component blend (P=0.091). False trail following was unlikely to be as important as habituation or sensory overload, as the best formulation was only weakly attractive compared to virgin females.

Keywords:*mating disruption*

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S608F01

Behavior and Chemical Ecology

Identification and assessment of a botanical termiticide from *Eremophila mitchellii*

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The wood of false sandalwood, Eremophila mitchellii, is resistant to termite attack in its natural environment; however, the properties conferring this resistance were unknown. Our research established that the essential oil from the heartwood of this species provides much of this resistance. Steam-distilled E. mitchellii wood oil exhibited toxic, repellent and antifeedant activity against the termites Nasutitermes exitiosus and Coptotermes acinaciformis in the laboratory. Detailed analysis of the oil was carried out by GC-FID, GC-MS, LCMS and NMR spectroscopy. After fractionation by preparative HPLC, eight components of the wood oil were characterized and accounted for >80% of the oil. Bioassay-guided fractionation determined that eremophilone and 8-hydroxy-1(10)- dihydroeremophilone were the principal active constituents in the oil. Pinus radiata wood impregnated with E. mitchellii oil was found to reduce damage by C. acinaciformis and Mastotermes darwiniensis in the laboratory, and by C. acinaciformis in the field.

Keywords:*botanical termiticide, repellent,*

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Combined neem oil and eucalyptus oil lotion: In vitro and in vivo efficacy study for the treatment of head lice

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Biomimic Lice Lotion, an intermediate polarity formulation comprised of the natural products neem oil and eucalyptus oil, was tested for its pediculicidal and ovicidal efficacies in the treatment of head lice (Pediculus humanus var. capitis) infestations. Initial in vitro testing demonstrated pediculicidal and ovicidal efficacies of 95.7% and 100% respectively. A follow up clinical study of 45 female school children, with two lotion applications one week apart, showed significant in vivo activity. In the clinical study, pediculicidal efficacies of 92.9% and 96.6 % were measured two days after the first and second application of the lotion respectively. Similarly, the lotion also showed a high ovicidal efficacy of 80% and 100% respectively after each application. A questionnaire conducted at the conclusion of the trial indicated strong acceptance of the formulation by the participants and their parents.

Keywords:*head lice, neem oil, pediculicidal, ovicidal*

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S608F03

Behavior and Chemical Ecology

Plant essential oils as repellents for agricultural pest management

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Numerous plant essential oils and some of their major constituents have been shown to be insecticidal and/or repellent to several important insect pests. In particular, small soft-bodied insects (aphids, psyllids, thrips, whiteflies) and spider mites are susceptible and field trials in several countries suggest that pest management products based on plant essential oils can be efficacious as crop protectants. Under field conditions it is difficult to unambiguously demonstrate repellence as a mechanism of action, whereas laboratory studies have been unequivocal in that regard. Given the volatility of many essential oils and their constituents, formulations and slow-release devices may be necessary to ensure adequate residual action as repellents. We have recently found that certain essential oils from Citrus species can prevent or delay the movement of the two-spotted spider mite between plants in a greenhouse when applied to strings connecting plants. As strings are often used in greenhouse vegetable production to support growing plants, treated strings could limit the spread of spider-mite infestations in commercial greenhouses.

Keywords:*essential oil, repellent, deterrent*

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S608F04

Behavior and Chemical Ecology

Bioactivity of extracts from *Tripterygium wilfordii* against insects

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Botanical pesticides provide an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduction of environmental impacts. The Chinese medicinal plant, Tripterygium wilfordii Hook., has been shown to be a valuable source of biological pesticides. Our team investigated the bioactivity of alkaloids extracted from the root bark of T. wilfordii against larvae of striped stem borer (SSB), Chilo suppressalis, and diamondback moth, Plutella xylostella. In choice and no-choice tests, the median antifeeding concentration (AFC50) of the alkaloids against newly-hatched and 3rd instar SSB larvae were 188.3 mg L-1 and 22.5 mg L-1, 217.2 mg L-1 and 46.1 mg L-1 , respectively. The alkaloids also caused obvious growth inhibition in the SSB larvae, with growth-inhibition rates of 26.7% and 88.79% when alkaloid concentrations were 25 mg L -1 and 100 mg L-1 , respectively. The median lethal concentrations (LC50) for larvae treated for 24 h and 48 h were 676.4 mg L-1 and 326.5 mg L-1, respectively. The alkaloids also showed insecticidal activity against P. xylostella. The AFC50 of the alkaloids against 2nd instar P. xylostella larvae were 42.44 mg L-1 and 46.6 mg L-1 in choice and no-choice test, respectively . The LC50 for was 388. 5 mg L-1 for larvae treated for 72 h. The median effective growth inhibiting concentration (EC50)of alkaloids against 2nd instar larvae was 123.3 mg L-1. These studies provided a theoretical basis for further exploitation of T. wilfordii as a bio-pesticide.

Keywords:*Tripterygium wilfordii, bioactivity, insects*

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Effect of guava fruit surface substances on oviposition behavior of oriental fruit fly (*Bactrocera dorsalis* Hendel)

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In order to investigate the role of host plant surface substances to oviposition behavior of oriental fruit fly (Bactrocera dorsalis Hendel), guava fruits removed surface substances and guava fruit surface substances extracts were tested to influence oviposition behavior of oriental fruit fly. The results showed that removing surface substance can significantly reduce oriental fruit fly choice rate to fruits and fecundity on guava fruit. Choice rate was lower than 50 % on each observation time, and the fecundity decreased rate was 26.34 %. Guava fruit surface substances extracts could attract oriental fruit fly and stimulate female adults to lay more eggs. The fecundity increasing rate of methanol extract to oriental fruit fly was 21.40 ± 0.70 %, this was more significantly than the fecundity increasing rate of dichloromethane extracts. The stimulatory activity of methanol extracts to oriental fruit fly was dose dependent. The oviposition percentage was from 8.44 % to 32.00 % when the methanol extracts concentration was used from 0 to 10 gfe/mL. We also found that oriental fruit fly could lay eggs only existing guava fruit flesh volatiles.

Keywords:*Guava, Surface Substances, Oriental Fruit Fly, Oviposition Behavior*

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Development and use of a natural plant extract (semiochemical) for the management of pests and beneficial insects on cotton cropping systems in Australia

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Cotton crops worldwide continue to face pest problems whether it is conventional or genetically engineered crops. While adoption of transgenic crops has resulted in the reduction of synthetic insecticide use against lepidopteran pests (eg Helicoverpa spp.), there have been rapid increase in populations of sucking pests because they are unaffected by the Bt toxin expressed in the transgenic crops. These pests continue to cause significant damage to both transgenic and conventional cotton crops. One of the approaches is the use of natural plant chemical compounds or plant extracts. Studies were undertaken between 2001-2010 to develop a semiochemical for integrated pest management (IPM) on cotton crops. An Australian Native plant code-named Plant X was identified as having behaviour modifying compounds that can potentially deter insect egg lay, feeding as well as cause direct mortality to pests. The Plant X bioactive compounds were formulated and assessed against pests in a series of field trials on cotton crops. The study showed that application of 2 L/ha or 2% v/v of the Plant X product reduced Helicoverpa spp. egg lay and controlled 1-3rd instar larvae. Also, the product reduced populations of adults and nymphs of Bemisia tabaci, Creontiades dilutus and Aphis gossypii. The product was also found to have no significant effect on predatory beetles, bugs, lacewings and spiders when applied at 1.0, 1.5 and 2.0 L/ha rates. Therefore, the use of Plant X (semiochemical) product will enhance conservational biological control, support IPM and reduce overall pesticide use against pests on cotton crops.

Keywords:*Helicoverpa spp., Bemisia tabaci (b type), Creontiades dilutus, Aphis gossypii, semiochemical, IPM*

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S701M01

Insect related Interactions at a Multi-trophic Ecosystem

Electrical penetration graphs during brief phloem punctures by aphid stylets

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EPG systems, Netherlands

Aphids often insert their stylets into sieve elements without subsequent typical phloem phase activities, phloem salivation and phloem ingestion, as reflected by EPG waveforms E1 and E2, respectively. Mostly the potential drop (pd) waveforms from a sieve element cannot be distinguished from pds in other cells, except for a few species, which show repetitive potential drops (r-pds). These regularly repeated r-pds are longer and show somewhat different waveform features, on which will be reported in this presentation. In addition to the waveform differences, the SE cells differ in an important aspect from non-SE cells, i.e. SE cells have no vacuoles. The normal pd may have a period during which the stylet tips are inside the vacuole. A vacuole tip position during pd sub-phase II-2, could explain that sub/phase has not be correlated to non-persistent transmission of plant viruses. This aspect will be focused as well.

Keywords:*stylet penetration*

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S701M02

Insect related Interactions at a Multi-trophic Ecosystem S701

Role of immune regulators in controlling aphid / plant interactions

Geraldine Dubreuil[1], Philippe Giordanengo[2], Didier Crochard[3], Emeline Deleury[4], Antonin Schmitz[5], Jean-Christophe Simon[6], Christine Coustau[7]

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A striking observation coming from the analysis of the pea aphid genome was the absence of some genes known to be involved in the immune responses of other insects. In contrast to this apparent limitation in the antimicrobial arsenal, we identified 6 genes coding for the cytokines MIFs within the A. pisum genome. In mammals, MIFs are key regulators of the innate immune response. To start investigating the role of ApMIFs in the aphid biology, we analyzed their expression and showed a negative correlation between ApMIFs expression levels and the presence, or the establishment of secondary symbionts. By contrast, ApMIFs expression levels increased upon infection with a parasitoid or a non-mutualistic bacterium, demonstrating that expression levels differentially vary during agonistic or antagonistic interactions. Surprisingly, one ApMIF was found expressed in the salivary glands raising the question of the role of this immune regulator in the aphid-plant interaction. We therefore investigated the effect of ApMIF expression knock-down on aphid feeding behavior. Interestingly, a 60% reduction in expression of members from the multigenic ApMIF family correlated with a significant disruption of the feeding behavior, as analysed by the electrical penetration graph technique (EPG). These results showed that the ApMIF multigene familly may be involved in the interaction with symbionts and that it plays a key role in controlling the ability of the aphid to properly exploit and feed on their host plant.

Keywords:*Aphids, Host-parasite interactions, host-symbiont interactions, feeding behavior*

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S701M03

Insect related Interactions at a Multi-trophic Ecosystem

Behaviour and physiology of aphids related to potato resistance

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Crop resistance to insect is seen as the best approach to reduce the use of insecticide in crop protection. Wild Solanum species are used as a source of resistance to aphids for the development of new cultivars. The study of aphids host selection behaviour on resistant germplasm provides insight on the mode of resistance present in the wild Solanum parent. This approach also led to redefine the role of xylem sap consumption in aphids as a strategy to regulate the osmotic pressure of the haemolymph.

Keywords:*behaviour, aphids, crop resistance,*

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S701M04

Insect related Interactions at a Multi-trophic Ecosystem S701

Unravelling the mechanisms of resistance to bluegreen aphid and pea aphid in the model legume *Medicago truncatula*

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Aphids, including the closely related bluegreen (BGA; Acyrthosiphon kondoi) and pea (PA; A. pisum) aphid, are important pests in agriculture. Resistance to BGA and PA has been introgressed into the Medicago truncatula variety Jemalong (A17) through recurrent backcrosses to create a new aphid-resistant cultivar Jester (91% identical to A17). Resistance to BGA in Jester is conferred by a dominant gene called AKR (Acyrthosiphon kondoi resistance) located on the short arm of chromosome three in a region rich in CC-NBS-LRR genes. PA resistance in Jester is conferred by a dominant gene, termed APR (Acyrthosiphon pisum resistance), which lies approximately 22.3 cM distal from AKR in a region dense in CC-NBS-LRR genes. Analysis of transcriptional changes in defence related genes representing various signalling pathways and transcription factor profiling showed clear differences in the response to BGA vs. PA. A17 has a moderate resistance to both BGA and PA compared to the highly susceptible accession A20. Quantitative trait loci (QTL) analysis using an A17 x A20 recombinant inbred line population revealed that one locus, which co-segregated with AIN (Acyrthosiphon induced necrosis) on chromosome 3, is responsible for the reduction of aphid biomass (indicator of antibiosis) for both PA and BGA, albeit to a lesser degree for PA than BGA. Interestingly, two independent loci on chromosomes 5 and 3 were identified for the plant biomass reduction (indicator of plant tolerance) by PA and BGA, respectively, demonstrating that the plants tolerance response to these two closely related aphid species is distinct.

Keywords:*aphid-plant interactions, aphid resistance*

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S701M05

Insect related Interactions at a Multi-trophic Ecosystem S701

Plant vigour and amino acid content and host utilisation by free-living eucalypt psyllids

Martin Steinbauer
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Psyllids exhibit either lerp-forming or free-living nymphal biologies. The nymphs of lerp-forming species are restricted in the tissues they can access to those immediately below their lerps. Consequently, the amino acid requirements of different species can either be met by females laying on expanding tissue (young leaves) or by nymphs altering already expanded leaves through the salivary enzymes they secrete into plant tissues (e.g. when on old leaves). Free-living psyllids appear to have circumvented such restrictions by laying into rapidly growing shoots which are presumably supplied with photosynthate already high in amino acids. Free-living psyllids may therefore provide models for lerp-forming species that prefer to oviposit on young foliage. The assumption that free-living psyllids exhibit a preference for high vigour plant parts because they are higher quality resources has not been examined (at least not for Australian taxa). I will present the findings of a study that compared two species of Ctenarytaina host specific for two species of eucalypt that differ in plant vigour and consider how they answer this assumption.

Keywords:*host plant vigour, host plant quality, amino acids, Ctenarytaina*

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Use of Fluorescence, a novel technique to determine reduction in feeding of phloemophagous insects when exposed to CyazypyrTM and other insecticides

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Phloemophagous insects are extremely economically important pest in the multiple crops in the World. Growers in many places rely on the use of insecticides to reduce populations of these insects. However, insecticides may take a few days to cause insect mortality and some do not reduce feeding. Reducing feeding of whiteflies and aphid would decrease the physiological effects on plants as well as the sooty mold and potentially the transmission of viruses. Measuring the reduction in feeding after the exposure of Bemisia.tabaci and Myzus persicae to an insecticide has proven difficult. A novel technique was developed to assess the effect of insecticides in the feeding of these phloemophagous insect species. A series of laboratory experiments demonstrate the usefulness of fluorescence in determining B. tabaci and M. persicae feeding cessation. Fluorescein sodium salt is systemically absorbed by plants and also by the insects feeding on these plants. Insects start fluorescing shortly after the plant root system is submerged in the fluorescein sodium salt. The effect of three insecticides (CyazypyrTM, imidacloprid and spirotetramat) with different modes of action on the reduction in feeding of these phloemophagous insects was evaluated and compared using this novel technique. It was determined that B. tabaci nymphs and M. persicae feeding on a plant treated with CyazypyrTM have a significant reduction of feeding when compared to nymphs feeding on plants treated with imidacloprid and spirotetramat.

Keywords:*whitefly, aphids, Benevia, Cyazypyr, Exirel*

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S701M07

Insect related Interactions at a Multi-trophic Ecosystem S701

Resistance of sugarcane, *Saccharum* spp., to the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), the vector of *Sugarcane yellow leaf virus*

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The sugarcane aphid Melanaphis sacchari (Zehntner) (Hemiptera: Aphididae) is the main vector of the *Sugarcane yellow leaf virus* (SCYLV; genus Polerovirus, family Luteoviridae), a disease of economical importance in the sugarcane growing area. Resistance was detected in the sugarcane cultivar R 365, using a three-year field trial in La Reunion island. In laboratory, R 365 reduced aphid populations on potted plantlets and excised leaves. Using the electrical penetration graph technique, we detected a delayeddelayed aphid salivation in phloem and inhibition of passive phloem sap uptake in R 365. Future research to evaluate the resistance durability and efficiency against Melanaphis sacchari diversity and its potential for reduction of SCYLV incidence through sugarcane cultivation schemes will be discussed

Keywords:*Saccharum spp., aphids, antibiosis, antixenosis, EPG*

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S701M08

Insect related Interactions at a Multi-trophic Ecosystem

Cross talks between plant and insects can be perceived by infecting viruses: Putative ecological consequences

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Most plant viruses are transmitted by vectors, particularly by hemipteran insects. The interactions between plants, viruses and insect vectors are obviously highly sophisticated, but they remain only very fragmentarily understood. Indeed, while countless reports have focused on two way interactions between the couples plant-insect, plant-virus, and virus-insect, the studies actually addressing three ways interactions in this type of biological system remains extremely scarce. The impact of plant viral-infection on the insect behavior have sometimes been addressed, reporting for example increased insect attraction due to changes in leaf colors, in volatile emission, or in sap composition. Surprisingly enough, despite the fact that common defense pathways are induced in plants by viral infection and by insect attack, a possible impact of the insect colonization of the plants onto the viral infection cycle has never been addressed. We have recently demonstrated that Cauliflower mosaic virus (CaMV) is able to highjack the plant perception of aphid feeding, and to immediately react by producing transmission specific morphs within infected plant cells. Interestingly, we demonstrated that non-aphid stresses such as wounding, or exposure to CO2-saturated atmosphere is provoking the same CaMV reaction, correlatively enhancing the efficiency of vector-transmission. This discovery is opening a wide new horizon for research in this field, evaluating the possible range of viral reactions to the feeding of insect vectors, and the range of unrelated biotic or non-biotic stresses that can "falsely" provoke these viral reactions and increase transmission.

Keywords:*plant virus, vector transmission, aphid, plant stress, feeding behavior*

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S701M09

Insect related Interactions at a Multi-trophic Ecosystem S701

Characterising interactions between the common brown leafhopper *Orosius orientalis* and selected nutritional sources

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Orosius orientalis (Hemiptera: Cicadellidae), he common brown leafhopper, is a polyphagous vector of a range of economically important pathogens, including phytoplasmas and viruses, which infect a diverse range of crops. This polyphagous pest affects a range of economically important crops yet fundamentals of its feeding behaviour have been largely unexplored. Studies on the plant penetration behaviour by O. orientalis were conducted using the electrical penetration graph (DC-EPG) technique to assist in the characterisation of pathogen acquisition and transmission. EPG waveforms representing different probing activities were acquired from adult O. orientalis probing in planta, using two host species, tobacco Nicotiana tabacum and bean, Phaseolus vulgaris, and in vitro using a simple sucrose-based artificial diet. Five waveforms (O1-O5) were evident when O. orientalis fed on bean, whereas only four waveforms (O1-O4) and three waveforms (O1-O3) were observed when the leafhopper fed on tobacco and on the artificial diet, respectively. Both the mean duration of each waveform and waveform type differed markedly depending on the food substrate. Waveform O4 was not observed on the artificial diet and occurred relatively rarely on tobacco plants when compared with bean plants. Waveform O5 was only observed with leafhoppers probing on beans. The attributes of the waveforms and comparative analyses with previously published Hemipteran data is presented and discussed, but further characterisation studies will be needed to confirm our suggestions.

Keywords:*Orosius, Leafhopper, Vector, electrical penetration graph*

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S701M10

Insect related Interactions at a Multi-trophic Ecosystem

Impact of leafhoppers that vector phytoplasma in oilseed crops in the Canadian prairies

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The main oilseed crop grown in Western Canada is canola (mostly Brassica napus). False flax (Camelina sativa) is considered to be an alternative oilseed platform for the prairies due to its resistance to drought and to most diseases and insect pests. Aster yellow (AY) is caused by the phytoplasma species 'Candidatus Phytoplasma asteris', which is transmitted mainly by leafhoppers. In canola and false flax, AY is generally considered of little significance, with overall incidence less than 1%. However, over the past decade, several epidemics occurred with incidence reaching 40% in some fields. Plants and leafhoppers were sampled in camelina and canola fields throughout Saskatchewan (Canada) between 2001 and 2011. Disease incidence and yield losses were determined and phytoplasma infecting plants and insects were identified using PCR tests and DNA sequencing. No variation in disease incidence was observed between canola cultivars. However, camelina germplasms exhibiting no AY symptom were detected. The presence of phytoplasma DNA in canola and camelina seeds and seedlings will be discussed.

Keywords:*Camelina phytoplasma disease incidence*

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Insect vectors of plant pathogens threatening agricultural production in Sudan

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Several biotic and abiotic factors contribute to spreading of plant diseases and their transmissions among different host plants are well documented. However, insects are important biotic agents causing widespread distributions and outbreaks of many pathogenic infections in the field. For instance, the repeated epidemic outbreaks of the leaf curl viral disease in cotton and tomato crops are merely correlated to the high activities of Bemisia tabaci populations in different countries. Accordingly, this paper sheds light on the important species of insects involved in transmitting deleterious plant pathogens of economic significance in Sudan. About fifteen insect species (pests and non pests) reported as vectors of more than twenty plant pathogens, mostly viruses, were listed. Some insect pests, such as B. tabaci, Aphis craccivora and Aphis gossypii, were known to transmit complex of over five different destructive plant viruses, including leaf curl, mosaic, stunting, wilting, mottling, rosette and vein thickening viruses. Moreover, the magnitudes of damage inflicted on some major host plants were also outlined. Hence, some control measures proved to be successful against certain vectors in the country were suggested to be integrated for their management.

Keywords: *Vectors, plant pathogens, damage, host plants, Sudan.*

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S701M12

Insect related Interactions at a Multi-trophic Ecosystem S701

Investigating the impact of elevated CO2 on wheat, Barley yellow dwarf virus and its aphid vector *Rhopalosiphum padi*

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Climate change is a concern globally due to a possible impact on the environment and especially on agriculture. Predicted changes in CO2 and temperatures may have a major impact on agricultural systems. Nevertheless, there is a lack of empirical data on the effects of elevated CO2 and temperature on agricultural pest and pathogen populations. Consequently, predictions on the future of our major monoculture crops such as wheat, have not been fully understood. In a joint effort, the Department of Primary Industries Victoria, the University of Melbourne and the Department of Climate Change have established a Free-Air CO2 Enrichment (FACE) facility at Horsham, Victoria to study the effects of elevated CO2 on wheat production in Australia. The FACE facility in Horsham is being used to study the effects of projected CO2 concentrations (550ppm) under field conditions on wheat, the Barely yellow dwarf virus (BYDV) as well as the population dynamics and biology of its aphid vector Rhopalosiphum padi (Homoptera, Aphididae). Results from our studies on wheat plants under FACE conditions showed positive response to elevated CO2. However, variable field conditions have proved problematic for studying the impact of elevated CO2 on BYDV and its vector, thus in addition, controlled environment growth chambers were used to study in detail, the physiology and feeding behaviour of R. padi and its ability to acquire and transmit BYDV under different CO2 concentrations. Results from both the FACE and growth chamber experiments will be presented and the potential ecological and epidemiological consequences discussed.

Keywords:*BYDV, climate change, aphid, wheat*

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S701M13

Insect related Interactions at a Multi-trophic Ecosystem S701

Vegetable oil for PVY control

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Potato virus Y (PVY) is a phytopathogen virus transmitted in a non-persistent manner by aphids to a broad range of crops. Mineral oil application has been considered for several decades as an effective mean to protect plant against virus damages. Recently we demonstrate that mineral oil has no negative effect on insect fitness or its feeding behaviour but affect mainly the vector virus acquisition step. Furthermore we show that the treatment of potato plant by rapeseed oil prior to viruliferous aphid infestation, can also lead to a reduction in PVY transmission. In order to propose an alternative to mineral oil treatment, we investigated the effect of rapeseed oil treatment on potato plants on the feeding behaviour and fitness of Myzus persicae (Hemiptera, Aphididae). The electropenetrography technic is used to point out feeding item link to acquisition. We demonstrate divergent effects of plant treatment (oil-treated or not) and virus infection (PVY infected or not) on aphid feeding behaviour, growth and reproduction. This work brings new insights into our understanding of mineral and vegetable oils mode of action and more generally of the mechanisms governing the PVY epidemiological dynamics.

Keywords:*aphid, solanum tuberosum, electrical penetration graph, EPG, feeding behaviour, rapeseed oil*

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S701M14

Insect related Interactions at a Multi-trophic Ecosystem

Aphids acquired symbiotic genes from bacteria

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Aphids harbor an obligate mutualist, Buchnera aphidicola (Gammaproteobacteria), within the cytoplasm of specialized cells called bacteriocytes. Since the initial infection in a common ancestor of aphids more than 100 million years ago, Buchnera have been subjected to strict vertical transmission through host generations, and the mutualism between Buchnera and their host has evolved to the point that neither can reproduce in the absence of the other. During the course of coevolution with the host, Buchnera has lost a number of genes that are considered essential for bacterial existence. The genome of Buchnera from the pea aphid, Acyrthosiphon pisum, encodes about 620 genes (genome size: 650 kb), which is only one seventh of that of most related free-living bacteria, such as Escherichia coli. Our studies including genomic and transcriptomic analyses revealed that the pea aphid acquired 12 genes/gene fragments from bacteria via lateral gene transfer. Buchnera was the apparent source of two highly truncated pseudogenes. Most other transferred genes were closely related to genes from relatives of Wolbachia (Alphaproteobacteria), which are absent in the analyzed aphids. At least eight of the transferred genes appear to be functional, and seven are highly transcribed in bacteriocytes. The results excluded the hypothesis that genome reduction in Buchnera has been accompanied by gene transfer to the host nuclear genome, but strongly suggest that aphids utilize a set of genes transferred from other bacteria in the context of the Buchnera?aphid mutualism.

Keywords:*aphid, Buchnera, bacteriocyte, symbiosis, lateral gene transfer, Wolbachia*

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S702M01

Insect related Interactions at a Multi-trophic Ecosystem S702

Predicting the effects of climate change on mealybugs and their natural enemies in grapevines in Australia

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Pest damage will depend on individual pest effects, changes in pest complexes and relevant natural enemies. We looked at potential for change in the relative importance of mealybug species in Australian vineyards. Distributions of two commonl native species longtailed mealybug Pseudococcus longispinus (Targioni Tozzetti), and citrophilus mealybug Pseudococcus calceolariae (Maskell) were mapped from pest records and potential responses under climate change modelled using MAXENT. Lethal temperatures were determined in our laboratory by subjecting individuals to temperature treatments for 2 h and compared to available data for a potentially invasive, vine mealybug Planococcus ficus (Signoret) taken from published limits. Vine mealybug, while currently not present in Australia, has shown itself to be an efficient invader, now a key pest in Europe, Africa, South Africa, Argentina, the Middle East, California and Mexico, and currently regarded as a potential threat to the Australian industry. Vine mealybug has a number of traits that may make it particularly damaging and difficult to control, including a potential for faster population growth resulting from more generations per year and ecological changes. It may also be a more efficient transmitter of grapevine leafroll viruses, a cause of increasing concern in grape growing regions globally. Of the many natural enemies of mealybugs, we looked at thermal responses of a predator, ladybird beetle Cryptoleamus montrouzieri Mulsant and a parasitoid Leptomastix dactylopii Howard. Responses of the pest and natural enemies were compared to investigate potential changes in the control efficacy of the natural enemies through changes in their distribution and abundance.

Keywords:*vine mealybug, longtailed mealybug, ladybird beetle, citrophilus mealybug, parasitoid*

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S702M02

Insect related Interactions at a Multi-trophic Ecosystem S702

Trophic interactions along climatic gradients with *Neodiprion sertifer* as a model species

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A frequently recurring question is if problems with insect pests in forests and agricultural crops will become more intense in a future, warmer climate. Higher temperatures are favorable for insects by increasing their activity and shortening the development time, thereby reducing the time exposed to natural enemies. On the other hand, higher temperatures will also affect the natural enemies and the host plant of the insect, and as a consequence change the relative importance of the mechanisms regulating the population. By studying temperature effects on interactions driving population dynamics we hope to become able to predict the future performance and outbreak risk of pest insects. We have studied the effect of temperature on the performance (survival, development time and weight) of a forest defoliating insect, the European pine sawfly, Neodiprion sertifer (Hymenoptera: Diprionidae) in the laboratory and in a latitudinal gradient in the field. Preliminary results suggest different responses to temperature from insect larval predators and mammalian pupal predators in this system, indicating that predation rate on larvae may increase while the pupal predation rates could decrease. The direct effects on the sawfly were as expected: higher temperatures reduced development time mainly in the younger larval stages and resulted in a lower final cocoon weight. Our results imply that the relative importance of different regulatory mechanisms for a species (e.g. food quality and predation by different types of natural enemies) may change with increasing temperatures and thereby affect the probabilities for outbreaks.

Keywords:*herbivore, forest pest, outbreak, sawfly, temperature*

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S702M03

Insect related Interactions at a Multi-trophic Ecosystem S702

Climate change impact on extrinsic and intrinsic competition between egg parasitoids

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Climate changing is a real phenomenon and the last decade was the warmest decade ever. The impact of climate change on animal communities is complex, affecting species interactions in all trophic levels, in different ways and in different bio-geographic zones. Here we will discuss about the possible effects of climate changes in shaping the competition between egg parasitoid species to consume the same hosts. Egg parasitoids are important biological control agents due to their ability to kill the pest before the crop-feeding stages emerge. Competitive effects can be divided in "extrinsic competition", the indirect interactions between adult females searching for hosts, and "intrinsic competition", the competition that occurs between larvae developing in the same host. The climate change impact will be addressed taking into account: 1) how quantitative and qualitative variation in plant volatile organic compound (VOC) emission may affect extrinsic competition; 2) how changes in host's biology may affect intrinsic competition as consequence of global warming. Finally data on intraguild interactions under global warming scenario between two sympatric egg parasitoids of a true bug will be presented.

Keywords:*species interactions, global warming, host location, multiparasitism*

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S702M04

Insect related Interactions at a Multi-trophic Ecosystem S702

Efficiency of volatile multitrophic signals under changing environmental conditions

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Plants produce and emit a complex array of volatile organic compounds (VOCs). The blend of these chemicals is substantially altered depending on the physiological state of the plant. Feeding by herbivores, infection by pathogens and numerous abiotic stressors elicit quantitative and qualitative changes in the volatile chemical blend. Numerous interactions between plants and their community members are mediated by these chemicals and are often specific to the stress induced chemical blend. These interactions include the attraction or repulsion of herbivores, the attraction of predators and parasitoids and plant-plant communication. One of the biggest challenges to the field of chemical ecology is the interpolation of laboratory collected behavioural data to the larger ecological scale. At this juncture, plants are exposed to combinations of abiotic factors, which may stress plants or act directly on the volatile chemical messengers that they emit. Ground-level ozone, concentrations of which have risen over the course of the last century, is one such factor. Ground-level ozone exerts oxidative stress on plants and induces the emission of VOCs. In addition, it breaks down numerous VOCs, altering the composition of the chemical blend and reducing the distance over which it remains intact. In this talk I will discuss the implications of changing environmental conditions on the volatile-mediated interactions occurring in multitrophic systems.

Keywords:*Environment, multitrophic interactions, ozone, volatile organic compounds, herbivore, Predator, plant-plant signalling*

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S702M05

Insect related Interactions at a Multi-trophic Ecosystem S702

Invaders, parasitoids and cyclic resident dynamics - A fatal equation?

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The mountain birches in boreal Fennoscandia have, for centuries, been periodically defoliated by a resident geometrid moth, Epirrita autumnata. The densities at the peak phases of the cyclic population fluctuations may lead to death of vast birch forest areas. Recent empirical studies have confirmed the importance of parasitoids in driving these population cycles. The global warming, has, however, brought a new factor to the complex moth-parasitoid dynamics as another cyclic geometrid, Operophtera brumata, has expanded its outbreak range to Finnish Lapland. This closely related invader has spread due to warming winters and has already caused severe defoliation and tree deaths on the host it shares with the resident E. autumnata. In addition, the two moths have common parasitoids in these areas. Asymmetric preferences of both parasitoids and predators have been observed between the moth species in earlier studies. As invasive species can have profound effects on resident community via indirect trophic interactions, it is important to study the consequences of asymmetries on population level. Observing the effects may, however, be difficult when studying cyclic populations. A modelling approach was therefore used to see the potential consequences of the recent invasion on resident population dynamics. Utilising empirical data from northern Fennoscandia, we modelled the invasion to the ecosystem. The coexistence of the two moth species and effects of indirect interactions on the long term population dynamics were then analysed. Independent of the modelled dynamics, the invasive species was observed have the potential to inflict drastic changes in the mountain birch community.

Keywords:*adaptive dynamics, asynchronic cycles, invasive species, mathematical modeling*

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Constant versus fluctuating temperatures in the interactions between *Plutella xylostella* and its larval parasitoid *Diadegma insulare*

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Using three constant (7, 22 and 30°C) and three corresponding fluctuating temperatures (0-14, 15-29 and 23-37°C), laboratory studies were conducted to determine the effects of different temperature regimes on the interactions between the insect pest, diamondback moth (DBM), Plutella xylostella (Lepidoptera: Plutellidae) and its parasitoid Diadegma insulare (Hymenoptera: Ichneumonidae). Parasitized third instar DBM larvae were reared until adult mortality in different temperature regimes in individual thermal gradient cells. Different biological parameters, including larval mortality, successful parasitisation rate, pupal mortality, larval and pupal development time, adult longevity, pupal weight, pupal shell weight, and adult dry weight were recorded. The highest parasitism rate (70%) was found at temperature regimes of constant and fluctuating 22°C and fluctuating 7°C, and the lowest parasitism was (30%) at fluctuating 30°C. At all temperature regimes, the mortality of D. insulare pupae was higher than that of DBM pupae; these differences were larger at higher temperatures. There were longer development times and higher pupal body masses at lower temperatures for both insects. Fluctuating compared to constant temperatures caused shorter development times, similar body mass and higher adult longevity for both insects in optimal and lower temperature ranges; however, fluctuation around the highest temperature did not cause such consistent patterns. Therefore, fluctuating temperatures as found in nature must be taken into account when conducting thermal studies in the laboratory. Comparing biological parameters of host insect and parasite in different temperature regimes, we conclude that Diadegma insulare is a more competent parasitoid at lower temperatures.

Keywords:*Plutella xylostella, Diadegma insulare, biological control, fluctuating temperatures, interactions, climate change*

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S703TU01

Insect related Interactions at a Multi-trophic Ecosystem

Host plant shift caused loss of a biosynthetic enzyme in larvae of *Chrysomela lapponica* (Chrysomelinae)

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Leaf beetle larvae of the subtribe Chrysomelina are able to exploit secondary metabolites from their host plants to create their own chemical defence. The metabolites are transferred from the larval gut lumen into the defensive secretion for further conversion into the deterrents. The ability for sequestration is already present in deterrent de novo-producing larvae and we demonstrated that exogenous compounds can interfere with the enzymes of the de novo-synthesis. In contrast to the de novo producers, the sequestering species specialized more and more in their host plants, e.g. salicin sequestration requires feeding on salicaceous plants. But in the course of Chrysomelina evolution host shifts occurred from Salicaceae to Betulaceae especially in the species Chrysomela lapponica. These shifts are mirrored in the secretion composition: willow feeders produce manly salicyl aldehyde and birch feeders mainly a cocktail of different butyryl esters. By secretome analyses we revealed that the lack of salicyl aldehyde is due to the loss of the functional enzyme salicyl alcohol oxidase (SAO) which catalyzes the oxidation from the alcohol to the aldehyde. A mutation in the SAO gene is responsible for the loss of SAO activity in the secretion of birch feeders [1]. [1] R. Kirsch, H. Vogel, A. Muck, J.M. Pasteels & W. Boland. Host plant shifts affect a major defense enzyme in Chrysomela lapponica. (2011) Proc. Natl. Acad. Sci., USA., 108, 4897-4901.,

Keywords:*Chrysomela lapponica, host plant shift, Salicylaldehyde oxidase*

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S703TU02

Insect related Interactions at a Multi-trophic Ecosystem

Precise RNAi mediated silencing of metabolic active proteins in defense secretions of juvenile leaf beetles

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Insects are extraordinarily inventive when it comes to producing defensive compounds for repelling their enemies. To circumvent auto-intoxicative effects, these natural products frequently originate in the epidermis derived exocrine glands. The gland cells produce secretions that are fortified with defensive compounds. Final conversion of non-active precursors into allomones in the secretions enables production of otherwise cytotoxic or instable compounds. For example, the juveniles of the mustard leaf beetle Phaedon cochleariae synthesize bio-active iridoids from non-toxic glucosides in their discharges. We have started with proteome analyses and identified putative proteins from about 25 families in the larval secretions. Next, we have utilized for the first time RNA interference (RNAi) to analyze in-vivo functions of enzymes in biosynthetic pathways in defensive secretions from insects. The iridoid biosynthesis proceeds from 8-hydroxygeraniol-8-O-β-D-glucoside to 8-oxogeranial, and a predicted enzyme with cyclase activity subsequently forms iridoids in the discharges. We have identified a novel protein belonging to the Juvenile Homone Binding Protein superfamily which is involved in the cyclization reaction of monoterpenes made clear by accumulation of the acyclic C10-precursor and simultaneous decrease of the methylcyclopentanoid monoterpenes five days after triggering RNAi. Cyclisation of monoterpenoids is not only known from insects but also plants use this yet enigmatic mechanism for the production of indole alkaloids. By routine usage of RNAi we found additional enzymes involved in the iridoid pathway but also a super oxide dismutase, the silencing of which did not affect the iridoid synthesis. Its function in defensive secretions is subject of current research.

Keywords:*leaf beetle, defensive secretions, iridoid metabolism, RNAi*

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Effects of floral volatiles on plant indirect defense against herbivory: Flowering transgenic torenia plants as a new tool

Takeshi Shimoda
National Agricultural Research Center, Japan

Plants under herbivore attack emit a blend of herbivore-induced plant volatiles (HIPVs) that can attract carnivorous natural enemies of the herbivores. Composition of HIPV blends may be important in plant defense against herbivores, because HIPV blends vary according to plant and herbivore species, and because different HIPV blends can attract different natural enemies. Furthermore, floral volatiles may also affect the effectiveness of plant indirect defense via HIPVs. To test this we I investigated the abilities of transgenic, wishbone flower plants (Torenia hybrida and T. fournieri) infested with spider mites (Tetranychus urticae), emitting a trans-volatile [(E)-β-ocimene] in the presence or absence of endogenous volatiles (natural HIPVs and/or floral volatiles), to attract predatory mites (Phytoseiulus persimilis). P. persimilis females were attracted to natural HIPVs from infested wild-type (wt) plants of T. hybrida but not to those of T. fournieri. The predatory mites preferred novel HIPV blends from infested, transgenic T. hybrida to natural HIPV blends from infested, wt T. hybrida. In contrast, such olfactory discrimination between transgenic and wt plants was not observed in T. fournieri. These results suggest that (E)-β-ocimene enhanced the predator attractiveness of HIPVs blends, but did not so when original HIPVs were non-attractive. Intriguingly, floral volatiles abolished the enhanced attractive ability of T. hybrida transformants, although the floral volatiles themselves did not elicit any attraction or avoidance behavior. These results suggested that the entire blend of plant volatiles including (E)-β-ocimene is important for attracting P. persimilis.

Keywords:*Torenia, (E)-β-ocimene, herbivore-induced plant volatiles, transgenic plant, predator attractiveness*

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S703TU04

Insect related Interactions at a Multi-trophic Ecosystem

Microorganisms in spider mites (*Tetranychus urticae*) regulate ecological interactions with lima bean plants

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In response to herbivory, plants emit specific blends of volatiles that attract carnivorous natural enemies of herbivores. These volatiles are called herbivore-induced plant volatiles (HIPVs). HIPVs emitted from two-spotted spider mite (Tetranychus urticae)-infested lima bean plants attract predatory mites (Phytoseiulus persimilis) that prey on the spider mites of genus Tetranychus. Here, we hypothesized that microorganisms in the spider mites were involved in the induction of a specific blend of HIPVs since production of HIPVs induced by the spider mites is involved in salicylic acid (SA)-related signaling pathway in lima bean plants. Microorganisms in the spider mites were divided into two types. One is exo-microbes which exist on the surface or in the gut of the spider mites. The other one is endo-microbes like intercellular symbionts. Recently, it has been reported that intercellular symbionts of insects regulate metabolism and behavior of their hosts. To address the hypothesis, we prepared the following three types of spider mites: exo-microbes-free mites, endo-microbes-free mites and both microbes-free mites. We compared the responses of lima bean plants to damage caused by either type of spider mites. Our results suggest that exo-microbes and endo-microbes in the spider mites are involved in the induction of specific blend of HIPVs through the activation of SA-related signaling pathway in lima bean plants.

Keywords:*herbivore-induced plant volatiles (HIPVs), intercellular symbiont, lima bean, predatory mite (Phytoseiulus persimilis), salicylic acid, two-spotted spider mite (Tetranychus urticae)*

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S703TU05

Insect related Interactions at a Multi-trophic Ecosystem

Interactions between tomato volatile organic compounds and aphid behaviour

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In the system constituted by tomato, Lycopersicum esculentum (L.), the aphid Macrosiphum euphorbiae (Thomas) and its natural enemy Aphidius ervi (Haliday) it has been showed that the release of volatile organic compounds following aphid attack is responsible for the attractiveness towards the aphid parasitoid in wind tunnel experiments. The main compounds involved in these multitrophic interactions have been characterized and quantified. The possible activity of these compounds in inducing plant direct defences against the aphid M. euphorbiae has been assessed in laboratory tests. The selected VOCs have been applied to uninfested tomato plants either by evaporation or contact and performances of aphids, in terms of plant acceptance, fixation behaviour and aphid development, calculated in standard conditions. The results showed that compounds involved in parasitoid foraging behaviour exhibit a direct effect on aphid performances.

Keywords:*Aphidius ervi, Macrosiphum euphorbiae, indirect defences, direct defences*

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S703TU06

Insect related Interactions at a Multi-trophic Ecosystem

Effects of plant-plant signaling on the colonization and the diversity of arthropods on willow plants

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A plant damaged by herbivores emits a blend of volatiles that is quantitatively and/or qualitatively different from that emitted from an intact conspecific plant. Such volatiles are known to induce defense-related genes in neighboring plants, and consequently enhance their defense against herbivores. This phenomenon is called a plant-plant signaling. Here, we investigated whether a composition of arthropods community on plants is affected by such a plant-plant signaling in nature. To test this, we used a willow plantation in Center of Ecological Research, Kyoto University. We placed pots of (1) intact willow plants, Salix eriocarpa (Salicaceae), exposed to volatiles from conspecific plants infested by leaf beetles Plagiodera versicolora (Coleoptera: Chrysomelidae), (2) willow plants infested by leaf beetles, and (3) intact willow plants (control). We counted the number of arthropods immigrated into plants with different treatments, and the number of leaves infested by herbivores (every day for one week, and then once a month for two month). The timing of immigration of adult leaf beetles, and the composition of arthropods were apparently different among three treatments. Species richness and abundance of arthropods on each treated and control plants were not significantly different. Interestingly, the number of newly emerged sprouts was higher in intact plants exposed to infested plant volatiles than in intact control plants. Such changes would affect arthropods' niches in a local plant community.

Keywords:*community assembry, plant-plant communication*

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S703TU07

Insect related Interactions at a Multi-trophic Ecosystem

Tritrophic interactions among tospovirus, thrips and Arabidopsis

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The western flower thrips (Frankliniella occidentalis) is a polyphagous herbivore that causes serious damages on many agricultural plants and also transmits tospoviruses, such as tomato spotted wilt virus (TSWV). Therefore, feeding damage and virus disease caused by thrips attack are serious problems in many countries. Our previous study reported that JA plays an important role to plant response and resistance to thrips, and JA-regulated plant defense decrease thrips performance and preference. In this meeting, we report the analyses of the tritrophic interaction between plants (Arabidopsis plants) and insect vector (western flower thrips), and also plant virus (TSWV). In ecological system, TSWV only move to the new host plants from the infected host plants by insect vector, thrips. We indicate that TSWV infection enhances thrips performance such as feeding activity and increases thrips population density of next generation in Arabidopsis plants. TSWV infection elevated Salicylic acid (SA) contents and induced SA-regulated gene expression. Meanwhile, TSWV infection decreased thrips feeding inducible JA-regulated gene expression. We also indicate that TSWV infection enhances the thrips preference of host plants. Thrips was attracted to the TSWV infected plants, and its attractance was decreased in JA insensitive coi1-1 mutants as compared to WT plants. In addition, SA application to WT plants enhanced this thrips attractance like TSWV infection. Our results suggest the mechanism of virus strategy to attract vector thrips to virus-infected plants taking advantage of antagonistic SA-JA plant defense system.

Keywords:*tritrophic interactions, plant defense*

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Contribution of metabolomics and transcriptomics to understanding of plant defense against herbivores

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A current progress and accessibility of molecular tools in plants, originally designed for Arabidopsis, allowed practical use of non-model plant species in biological research. Such models provide completely new plant properties and responses to be studied, and offer new ecologically relevant systems to be used in research of natural interactions. In particular, the combination of "omic" tools, such as transcriptomics and metabolomics, provided very useful method for reconstruction of plant signaling pathways and mechanisms that control accumulation of defense metabolites in stressed plants. We provide a practical example of gene expression profiling combined with metabolomics that was used to elucidate the role, biosynthesis and regulation of phenolamide secondary metabolites in defense of Nicotiana attenuata plants against specialist and generalist herbivores. Similar approaches are currently used in Japan to elucidate the innate signaling pathways and defense in monocots including rice plants.

Keywords:*phenolamide biosynthesis, tobacco defense, transcription factors, Nicotiana attenuata, Oryza sativa*

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S704W01

Insect related Interactions at a Multi-trophic Ecosystem

Ecological and evolutionary aspects of indirect interaction webs

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There is increasing appreciation of ecological consequences of plant-mediated indirect interactions between temporally-, spatially-, and taxonomically-separated species in a context of trait-mediated indirect interactions. In particular, herbivore-induced phenotypes of plants can form indirect interaction webs dominated by not only trophic but also nontrophic and indirect interactions. The phenotypic plasticity of terrestrial plants caused by herbivores is ubiquitous, and thus the plant-centered indirect interaction webs should be prevalent in a wide range of ecosystems. In addition to the species-level interactions, herbivore-induced plant phenotypes can initiate bottom-up trophic cascading effects on the associated community structure and biodiversity, leading to strong community-wide impacts on not only herbivores but also predators. This is because plant phenotypes induced by herbivores have the potential to increase variation and heterogeneity of resources that offer distinct niches for arthropods to coexist. Although such studies are still rare and enable very limited general conclusions, several trends emerge from the existing data. Variation in responses of species richness and abundance of higher trophic levels is largely dependent on the different ways in which plants respond to herbivores. On the other hand, community structure and biodiversity have the potential to indirectly promote trait evolution of community members through changes in plant phenotypic expression, which can impose selection on adaptive traits in insect herbivores. I will highlight the ecological and evolutionary consequences of plant-centered indirect interactions, to bridge the gap between evolutionary and community ecology.

Keywords:*indirect interactions, bottom-up trophic cascade, community structure, trait evolution*

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Root feeders and above-ground multi-trophic interactions

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It has been recognised for at least 20 years that aboveground and belowground insect herbivores can interact via induced changes to their shared host plants. Plant-mediated mechanisms, including changes in primary and secondary metabolites, are largely thought to underpin these interactions yet few studies have addressed both nutritional and defensive mechanisms simultaneously. Still fewer have attempted to investigate whether such interactions observed in the laboratory translate into changes in field populations of insect herbivores. Using a perennial woody crop, Ribes nigrum (blackcurrant), we have investigated the effects of root herbivory by Otiorhynchus sulcatus (vine weevil) on Cryptomyzus galeopsidis (blackcurrant aphid) and Nematus olfaciens (European blackcurrant sawfly). Performance of aphids increased in the presence of weevils, whereas sawfly performance decreased when sharing a plant with weevils. Increased aphid performance was linked to root herbivore induced increases in the proportion of essential amino acids, whereas poorer sawfly performance resulted from reductions in leaf phosphorus. Aphids induced higher levels of secondary metabolites (specifically phenolics) in roots, but O. sulcatus performance was not adversely affected. Field observations intermittently reflected these interactions; plants with O. sulcatus had fewer sawflies, but higher numbers of aphids. Aphid population build-up increased abundance of natural enemies, which reduced aphid populations to levels equivalent or less than weevil-free plants. To our knowledge, this is the first mechanistic example of plant-mediated effects of root herbivores on different aboveground feeding guilds to be demonstrated in both laboratory and field conditions.

Keywords:*aboveground-belowground, aphids, weevils, phosphorus*

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Breaking down plant defenses: Cross-talk mediating facilitation in inter-guild interactions

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Insect attack changes the physiological status of plants locally and systemically, extending the interaction beyond the two-species pair to the entire community of herbivores and their natural enemies. Most plant-mediated interactions among herbivores seem to result in competition, mediated by induced plant defences which negatively affect not only the initial attacker but also the subsequent ones. I will show a case study where insects from contrasting feeding guilds benefit from the distinct pathways triggered in the plants by them, representing a constraint for the plant when sequentially attacked by aphids and caterpillars. The observed facilitation was asymmetric; caterpillar performance was significantly higher on plants previously attacked by aphids, while aphids were not affected by the presence of caterpillars. The reduced levels of JA and low expression of well-known JA-biosynthesis and JA-regulated genes in systemic leaves of plants attacked by aphids suggest that the facilitation of caterpillar growth and development was mediated by interference with signal-transduction as a result of previous aphid feeding. The facilitation between the herbivores also had consequences for their parasitoids. Overall, both the caterpillar and aphid parasitoid benefited from the facilitation between their hosts. Neonate caterpillars, expected to be highly immobile with limited discrimination ability, proved to be active and selective and preferred to feed on plants with aphids compared to uninfested plants. Aphid infestation therefore turned the plants more susceptible and more attractive to caterpillars, and although counterintuitive, these effects were mediated by plant defenses. How widespread these interactions are, remains to be investigated.

Keywords:*plant-mediated interactions*

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S704W04

Insect related Interactions at a Multi-trophic Ecosystem

Localized plant induction: Impact on insect fitness and disease resistance

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Plants can respond to herbivore damage with phenotypic plastic changes in quality that negatively affect herbivores and prevent subsequent attack. These induced changes may be costly to implement and therefore, it may be more efficient for plants to respond locally, rather than systematically, to herbivore damage. However, studies focusing on localized induced resistance of trees in response to damage by herbivores are scarce. We evaluated the influences of localized induced changes in red alder trees in response to insect feeding on fitness correlates for both female and male western tent caterpillars (Malacosoma californicum pluviale). We infested selected branches of trees with tent caterpillar larvae under field conditions and maintained the neighboring branches as non-infested controls. In the laboratory, larvae were fed leaves from either "induced" or control branches through to the adult emergence. A second cohort of larvae was challenged with a viral pathogen to compare their disease susceptibility on induced vs. intact foliage. Herbivory induced localized responses in damaged branches that reduced leaf quality for growth and development of female western tent caterpillars but not males. However, larval survival was higher, and larvae fed induced leaves were more resistant to disease, than their counterparts on intact leaves. Our findings suggest that localized induced changes in leaf quality could potentially influence populations of tent caterpillars in contradictory ways by reducing their developmental rate and final size and therefore fecundity to a modest degree, while improving their survival and resistance to disease to a larger extent.

Keywords:*localized induced resistance, herbivore fitness, disease resistance, forest Lepidoptera*

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S704W05

Insect related Interactions at a Multi-trophic Ecosystem

Coping with a senescing environment: When insect reveal to plants the secret of eternal youth

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Phytophagous insects can be divided in two large groups according to their feeding habit: ectophages feeding externally on plant tissues and endophages living concealed within plant tissues and feeding internally. In spite of a reasonable understanding of the history and origins of the endophagous feeding habit, its evolution and adaptive significance remains unclear. Three major hypotheses have been proposed to explain the endophagous-feeding life history mode: The nutrition hypothesis, the microenvironment hypothesis and the enemy hypothesis. Among these hypotheses, feeding selectively on the most nutritional tissues is undoubtedly considered as a major advantage and this behavior can also be reinforced by manipulating the plant physiology as suggested by the autumnal formation of 'green-islands' around mining caterpillars in yellow leaves. Our results on the Malus domestica/Phyllonorycter blancardella plant-leaf mining system show the ability of leaf-miner insects to manipulate their host plant physiology and to create an "optimal" nutritional micro-environment through cytokinin production by their endosymbiotic partners. This symbiotic association impacts not only nutrient acquisition but also the feeding behavior of leaf miner insects and presumably allowed insects to specialize on specific host-plants. A close investigation at the interspecific level also shows a strong correlation between the presence of the endosymbiotic bacteria Wolbachia and the induction of green-islands suggesting that insect symbionts most probably played a major role in the evolution and/or radiation of the endophagous feeding mode.

Keywords:*plant insect interaction, plant manipulation, endosymbiont, Wolbachia, extended phenotype*

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S704W06

Insect related Interactions at a Multi-trophic Ecosystem

Biodiversity drives trait evolution of a community member in an indirect interaction web

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The dramatic loss of biodiversity has led researchers to pay increasing attention to the ecological consequences of biodiversity. However, no empirical studies have explicitly addressed a fundamental question how species diversity affects evolutionary responses of organisms in an ecological community, while the tight interactions between ecological community and organism evolution has recently been accepted. Interspecific interactions among herbivores mediated through host plant traits are often critical in herbivorous insect communities, in which prior damage by one herbivore species triggers plant phenotypic changes (e.g., induced defence) influencing growth, survival, and reproduction of another herbivore species sharing same host plants. Because a single plant species can show diverse ways of responses to attack by a wide range of insect herbivores, local species diversity of herbivore communities may affect expression of host plant phenotypes. Herbivorous insects may also locally adapt to such altered host plant phenotypes because insects have quite short lifetime compared to long-lived host plants. We show that difference in local species diversity of herbivore communities leads to divergent trait evolution of the leaf beetle (Plagiodera versicolora). Our field surveys and community-manipulated experiments demonstrated that local species diversity of herbivorous insects determined expression of host plant phenotypes through induced plant responses, resulting in differential selection regime for a feeding trait of the leaf beetle and among-population variation in the trait value. Consequently, herbivore species diversity can control evolutionary trajectories and genetic diversification of community members.

Keywords:*trait-mediated indirect effect, eco-evolutionary dynamics, diffuse selection, herbivore-induced plant response, willow, insect community*

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Sequential speciation amplifies biodiversity across a community of insect parasites.

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The role of species interactions across trophic levels can play a prominent role in fostering biodiversity. Diversity can beget diversity when speciation by one organism drives diversification of associated organisms in adjacent trophic levels, a term coined "sequential speciation". If organisms diverge and create new niches to exploit, catalyzing a "chain reaction" of speciation events, what are the frequency of these events and barriers to gene flow that reinforce diversification? The apple maggot fly, Rhagoletis pomonella (Diptera: Tephritidae), is a textbook example of incipient sympatric speciation. Flies within the R. pomonella complex (apple-, hawthorn-, blueberry- snowberry- and dogwood-infesting) are maintained by differences in eclosion timing, host odor preference and differ in allele frequencies. Recently the Rhagoletis?attacking parasitoid Diachasma alloeum (Hymenoptera: Braconidae), has undergone sequential speciation. Paralleling flies, host-associated populations of parasitoids exhibit differences in allele frequencies, eclosion timing, and odor preference. We tested if sequential speciation was applicable to the remaining members of the Rhagoletis?attacking parasitoid community (Diachasmimirpha mellea and Utetes canaliculatus). Following overwinter, mean eclosion times of both Diachasmimorpha and Utetes differ between host associations paralleling the fruiting phenology of host plants and the eclosion timing of fly hosts. In choice tests both species orients towards natal odors and avoid non-natal odors. Microsatellite loci exhibit consistent site-to-site host-related frequency differences in sympatric populations for both Diachasmimorpha and Utetes. Initial evidence supports sequential speciation across the community of Rhagoletis-attacking parasitoids supporting the hypothesis that sequential speciation is widespread among insect-parasite systems and contributes to the generation of biodiversity.

Keywords:*parasitoid, braconidae, Rhagoletis, sequential speciation, sympatry*

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Do plant-mediated interactions among herbivores affect parasitoid performance and genetic population structure?

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Individuals of different species interact in local communities and can influence each other's fitness both directly or indirectly through intermediaries. This is particularly true for the interactions among plants, insect herbivores and the natural enemies of these herbivores. It is hypothesized that herbivory by one insect will cause changes in a plant that will affect subsequent herbivores and their natural enemies. We test the importance of such effects for a multitrophic system comprising lima bean plants, several species of beetles, and their parasitoids. With this system we investigated the extent to which previous damage on bean leaves and seeds affects the performance and behavior of bruchid beetles and how such effects may cascade upwards to the next trophic level. Beans collected from different wild populations in Mexico were characterized for their content in cyanogenic glycosides. Of these, 4 populations were selected that varied significantly in their concentration of these glycosides and we examined the consequences of changes in plant allelochemistry due to attack by one herbivore on the performance of a second herbivore and its larval parasitoid. To mimic herbivory, we damaged the plants and treated them with jasmonic acid, a hormone implicated in induced defenses against insect herbivores. Our results support the hypothesis that prior induction of plant defenses as a response to damage inflicted to leaves and pods, will alter the suitability of seeds, resulting in a decreased performance of the seed beetle, Zabrotes subfaciatus and its parasitoid, Stenocorse bruchivora.

Keywords:*plant-mediated interactions, sequential herbivory, induced defenses, beans*

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S704W09

Insect related Interactions at a Multi-trophic Ecosystem

The role of host-plant and bacteria species in the genetic differentiation of herbivorous insects

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Host Associated Differentiation (HAD) is the formation of genetically distinct host associated populations. HAD has been invoked to explain the enormous diversity of phytophagous insects. Individual case studies of HAD are interesting but by themselves they are unable to provide clues on the mechanisms that might promote it. Thus, we are studying HAD on several species associated with the same host-plant species pair: Pecan and water hickory (both in the genus Carya). It has been suggested that bacteria in insect herbivores may play a role in HAD by allowing their insect hosts to use different plant species. The objectives of this study were: 1) to assess HAD in 6 Sternorrhyncha species associated with pecan and water hickory and 2) to document if HAD in these Sternorrhyncha corresponded with differences in the composition of their associated bacteria. HAD was determined using AFLPs and Bayesian clustering. Bacterial diversity was characterize through metagenomic analyses using 454 sequencing. HAD was present in 4 out of 6 species tested. Differences in bacterial diversity were found between pecan leaf Phylloxera populations associated with pecan and water hickory. The bacteria, Pantoea agglomerans and Serratia marcescens were absent in the Phylloxera water hickory population whereas both species accounted for more than 69.72% of bacterial abundance in the pecan population. Future studies will assess if the same phenomena occurs in the other Sternorrhyncha species in our system. The Carya system provides a parthenogen rich contrast to the already well characterized Solidago system for the study of HAD.

Keywords:*host-associated differentiation, HAD, bacterial symbionts, sympatric speciation*

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Host feeding by a gall-inducing insect improves the performance of offspring

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The maize orange leafhopper Cicadulina bipunctata (Hemiptera: Cicadellidae) induces galls characterized by stunted growth and severe swelling of leaf veins on various Poaceae. The gall induction by the leafhopper is different from that by other gall-inducing insects in that the galls are induced not on feeding sites but appear on distant, newly developing leaves. In addition, adult C. bipunctata is highly mobile and seldom feed on gall tissue that they have induced. These mean that the gall induction by C. bipunctata is unlikely to contribute to the fitness of the inducer itself. In this study we examined whether manipulation of the host plant by this leafhopper has a subsequent benefit to offspring or not. Adults feeding on maize seedlings caused a partial change in the glucose content and a remarkable change in the accumulation of free amino acids in the gall tissue. Increases in emergence and developmental rates were observed in nymphs feeding on gall tissue induced by prior adult feeding. Such improvements were not evident in nymphs feeding on a C. bipunctata-resistant variety, which rarely displays galls after C. bipunctata feeding, nor on maize seedlings previously foraged by another leafhopper, Psammotettix striatus. The results indicate that gall tissue induced by adult C. bipunctata contribute to better performance of its offspring through improvement of the nutritional components of host plants.

Keywords:*gall, leafhopper, Poaceae, Cicadulina, Cicadellidae*

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S705F02

Insect related Interactions at a Multi-trophic Ecosystem

Gall-inducing ability of the maize orange leafhopper *Cicadulina bipunctata* and barley chromosomes related to the resistance and susceptibility to the gall induction

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Although many herbivorous insects have the ability to manipulate host plants and induce galls for their own benefit, the mechanism of gall induction by insects is still unknown. This is partially because of the difficulty in establishing mass-rearing systems and performing comparative experiments using galling and non-galling relatives. The maize orange leafhopper, Cicadulina bipunctata (Hemiptera: Cicadellidae) is one of the suitable materials to clarify the mechanism. The leafhopper feeds on various poaceous plants and induces galls characterized by the swelling of leaf veins. A mass-rearing technique has already been established for this leafhopper, and model plants such as rice, wheat, and maize are available for experiments using the leafhopper. Moreover, some host plants such as barley and sorghum does not exhibit any symptoms even when they were fed on by the leafhopper. We examined the effect of barley chromosome addition to wheat on the susceptibility of wheat to C. bipunctata. As a result, barley genes resistant to gall induction were suggested to exist in chromosome 3. In contrast, wheat added barley chromosome 5 promoted gall induction by the leafhopper. In addition, we refer to geographical variations in the gall-inducing ability of the leafhopper and possible selection of non-galling strains.

Keywords:*gall, Poaceae, Cicadellidae*

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S705F03

Insect related Interactions at a Multi-trophic Ecosystem S705

Progress on gall midges (Diptera: Cecidomyiidae) in China

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The Chinese fauna of gall midges (Diptera: Cecidomyiidae) is rich and diverse with over 220 described species with about 180 nominal species in nearly 30-40 genera by our statistics, which are being used to complete one of our recent works: 'Catalog of gall midges (Diptera: Cecidomyiidae) in China: 1917 to 2012', by which many species recorded in older or scatter Chinese papers will come to light. And Chinese studies including our lab's work on advances in taxonomy, phylogeny, physiology, biology, ecology and biological control of gall midges are summarized and introduced to provide a reference for gall midge specialists worldwide further researching.

Keywords:*gall midges, China, Cecidomyiidae, progress, catalog, Chinese fauna*

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S705F04

Insect related Interactions at a Multi-trophic Ecosystem S705

Host specialisation, diversity and community structure of galling insects in a Papua New Guinean lowland rainforest

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In the Old World wet tropics, data on gall-forming insect communities are sadly lacking, especially in Afrotropical and Australasian/Oceanian regions. This is the first study to explore gall-forming insect communities and their parasitoid assemblages in a hitherto virtually unknown Papua New Guinean fauna. In order to test galler host specificity, plant phylogenetic indices were used, for the first time in a gall study, across a broad range of tropical rainforest trees. 32 species, representing 22 genera and 15 families of woody plants from all the major angiosperm clades, were sampled over an 8 month period in three lowland rainforest sites around Madang. All but three plant species hosted at least one out of 87 gall morphotypes from 1150 collections. More than 4000 insects were reared. At 2.7 morphotypes per host plant species, these data are comparable with other highly specialised folivores from the region: leaf miners & mesophyll suckers with 2.4 & 0.8 respectively. Initial findings reveal a community dominated by Cecidomyiidae (56%), followed by Hemiptera (26%), and Lepidoptera and Thysanoptera (6%), corresponding most closely with the Indonesian gall fauna. Approximately 96% of galls are estimated to be species specific in this study, the remainder being congeneric feeders. In this tri-trophic system, the host specialisation of the gall-formers' parasitoid assemblages will also be reported and discussed. This study will provide baseline data and pave the way for further investigation of gall-forming insect communities in Papua New Guinea.

Keywords:*Galls, host specificity, species richness, parasitoids, community ecology*

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S705F05

Insect related Interactions at a Multi-trophic Ecosystem S705

Carotenoids, lateral gene transfer and gall midges

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Major features of eukaryote genomes bear the mark of lateral gene transfer (LGT), with endosymbiosis being perhaps the most dramatic signature of large-scale LGT into diverse eukaryotic lineages. We report the discovery a rare example of fungal-to-animal transfer of carotenoid structural genes in gall midges (Diptera: Cecidomyiidae), joining aphids and two-spotted spider mites as the only animals know to possess the capability for in situ biosynthesis of carotenoids. Carotenoids are ubiquitous pigments with far-reaching roles in organismal biology, from central metabolism to communication. We describe patterns of molecular evolution in LGT carotenoids in gall midges with respect to recent studies on other arthropods. We report our progress on tracing the origins of carotenoid acquisition in flies and mapping their acquisition onto major evolutionary and phylogenetic features of the lower Diptera, and discuss how carotenoids may facilitate or inhibit antagonistic and mutualistic interactions in gall midges, and how they have opened new ecological opportunities and have spurred biological diversification in these flies.

Keywords:*carotenoids, diversification, plant-insect interactions, gall*

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Discovery of gall wasps (Hymenoptera: Cynipidae) inducing galls on the strictly Asian subgenus Cyclobalanopsis of the genus *Quercus* (Fagaceae)

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Of approximately 1,400 cynipid species described to date, about 1,000 species are oak gall wasps belonging to the tribe Cynipini. The host plants of Cynipini belong to the family Fagaceae, primarily the genus Quercus, but also Castanea, Castanopsis, Chrysolepis, and Lithocarpus. The genus Quercus is divided into two subgenera, Quercus and Cyclobalanopsis, with the latter subgenus being restricted to Asia. Despite the extensive distribution of Cyclobalanopsis in Asia, only two species of Andricus (Cynipini) described by Shinji had been recorded as gall inducers on this plant subgenus. However, these two species are actually inquiline gall wasps and were transferred to Ufo of the inquiline tribe Synergini. In 2010, Plagiotrochus masudai Ide, Wachi et Abe, which induces galls on Q. (C.) glauca Thunb., was described from Japan. This is the first definitive description of an oak gall wasp inducing galls on Cyclobalanopsis. After that, some cynipid species associated with Cyclobalanopsis were described from Japan and Taiwan one after another. In Japan, Synergus itoensis Abe, Ide et Wachi (Synergini) was described as a gall-inducing species in the acorns of Q. (C.) glauca. In Taiwan, another species of Plagiotrochus and four species of Cycloneuroterus were described. The latter genus was established in 2011 and named after the host association with Cyclobalanopsis. More recently, three species of Cycloneuroterus were also discovered from Japan. These findings may suggest the potentially diverse cynipid fauna on Cyclobalanopsis in Asia.

Keywords:*Cynipini, Asia, Plagiotrochus, Cycloneuroterus*

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S705F07

Insect related Interactions at a Multi-trophic Ecosystem S705

Inferring speciation history of the *Andricus mukaigawae* complex (Hymenoptera: Cynipidae) based on nuclear DNA and mitochondrial DNA Data

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The Andricus mukaigawae complex of oak gall wasps is composed of two cyclically parthenogenetic species, A. mukaigawae and A. kashiwaphilus, and a parthenogenetic species, A. pseudoflos. The component species differ in their life history, distribution, host plant, karyotype, asexual gall shape, and Wolbachia infection status although little difference is found in the external morphology of asexual adults. Therefore, among oak gall wasps, the A. mukaigawae complex provides a good opportunity for studies on the differences in host plants and in reproductive modes and on their relationship with genetic divergence. In order to understand the genetic relationship of the three species and the origin of asexuality, DNA sequences of one mitochondrial region and nine nuclear regions were investigated in multiple individuals from each of the three species of the complex. The genetic relationship among the three species suggested that a loss of sex had occurred after a host shift. Unexpectedly, two distinct groups were recognized in the parthenogenetic species A. pseudoflos by both the mitochondrial and nuclear DNA data. Gene flow in the nuclear genes from the Wolbachia-uninfected A. kashiwaphilus to infected A. mukaigawae was suggested by a method based on coalescent simulation, whereas gene flow in the mitochondrial genes was in the opposite direction. These findings suggest a possible involvement of Wolbachia infection in the speciation process of the A. mukaigawae complex.

Keywords:*gall-inducing insects, oak gall wasp, Wolbachia, gene flow, population structure*

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A koinobiont parasitoid manipulates its host cecidomyiid to modify gall morphology to avoid hyperparasitism

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Many parasitoids are known to manipulate host behaviour for their own benefit. Parasitoids of gall-inducing cecidomyiids are divided into two groups, early and late parasitoids, according to their parasitic strategies. Early parasitoids are usually koinobionts attacking eggs or first instars before maturation of galls in size. Late parasitoids are idiobionts attacking final instars or pupae by inserting their long ovipositors through the gall wall after maturation of galls. When early and late parasitoids attack the same host individual, only late parasitoid usually survive. Masakimyia pustulae Yukawa & Sunose (Diptera: Cecidomyiidae) induces dimorphic blister-like leaf galls (thick and thin types) on Euonymus japonicus and E. fortunei (Celastraceae). As a result of our survey, one platygastrid early parasitoid, Platygaster sp. (Platygastridae), and two eulophid late parasitoids, Chrysonotomyia sp. and Pnigalio sp. (Eulophidae), were found to parasitize M. pustulae. Among these parasitoids, the late parasitoids were frequently found in thin type galls, but seldom in thick type galls. This result indicates that the thick type gall is adaptive for avoiding parasitism by the late parasitoids. In either type, galls attacked by Platygaster sp. were significantly thicker than unparasitized galls in many localities. In addition, galls attacked by Platygaster sp. were significantly thicker than unparasitized galls from an early stage of gall growth. The percentage parasitism by the late parasitoids was significantly lower in galls attacked by Platygaster sp. than in those not attacked. These results strongly suggest that Platygaster sp. manipulates host behaviour to thicken galls to avoid hyperparasitism by the late parasitoids.

Keywords:*host manipulation, gall, late parasitoid, Platygaster sp., Masakimyia pustulae*

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Insect related Interactions at a Multi-trophic Ecosystem S705

Phylogeny and evolution of gall morphology in eriosomatine aphids

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Aphids of the tribe Eriosomatini (Aphididae: Eriosomatinae) are typically associated with the tree genera Ulmus and Zelkova (Urticales: Ulmaceae), on which they induce various types of leaf gall: open leaf rolls, or completely or incompletely closed pouch galls. To elucidate evolutionary change in gall morphology, phylogenetic relationships of the tribe Eriosomatini were inferred using 36 species (28 in the ingroup) and based on 52 morphological characters. Phylogenetic analysis with equal character weighting led to hundreds of most-parsimonious trees, and the strict consensus of these was poorly resolved. However, the successive weighting of characters yielded three most-parsimonious trees, and the strict consensus of these was well resolved. Ancestral reconstruction of gall morphology on the consensus tree suggests that the ancestral gall morphology was open leaf rolls, and that the completely and incompletely closed pouch gall evolved once each from the leaf rolls. The evolutionary transition from open leaf rolls to closed galls was also found in other galling insects, and this convergent evolution in different groups of galling insects suggests that closed galls may have adaptive advantages to the gall formers at the initial stage of its evolution, such as protection against general predators, gall inquilines and unfavorable abiotic conditions. However, it is likely that the restricted space in closed galls limits the number of aphids living inside, leading to the decrease of the number of generations in the galls.

Keywords:*adaptive advantage, ancestral reconstruction, life cycle, Ulmus, Zelkova*

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S705F10

Insect related Interactions at a Multi-trophic Ecosystem S705

Gall shape variation and host specificity in relation to species identity in a complex galling midge group *Daphnephila* (Diptera: Cecidomyiidae) on *Machilus* spp. (Lauraceae) in Taiwan

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Most of gall-inducers are monophagus or oligophagous, which utilize a narrow spectrum of host plants and organs. Diversity of gall inducers and the specific relationship between galling insects and host plants are considered as a consequence of adaptive radiation. Galls are then often regarded as extension of phenotypes of gall inducers. However, it is unclear to what extend could the shape variation of galls and host specificity represent species identity of gall inducers. Highly diverse group of gall midge Daphnephila spp. (Diptera: Cecidomyiidae) on Machilus (Lauraceae) in Taiwan are used to discuss this issue and multidisciplinary approaches were applied. Thirty-eight morphological species were recognized based on their gall shape, galling position, and host plant information. These galls with similar shapes could be further grouped into 8 series. Samples of gall midges induced similar shape of galls revealed monophyletic relationship based on molecular phylogenies with genetic differentiation ranging from 1.3% to 14.3% between groups. The gall shape series in general reflects the taxonomic status at the category of species or species group, whereas the gall morphospecies may not always reflect the species status. It is necessary to obtain evidences from different aspects and to integrate the overall variation for making correct taxonomic determination for the gall midges and henceforth elucidate their adaptation and sepciation.

Keywords:*gall morphology, host specificity, Daphnephila spp., Machilus spp.*

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Sexual isolation between two intraspecific groups of *Hartigiola faggalli* (Diptera: Cecidomyiidae) that induce different types of leaf gall on *Fagus crenata* (Fagaceae)

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Hartigiola faggalli (Diptera: Cecidomyiidae), a species that induces leaf galls on Fagus crenata (Fagaceae), was studied to assess the degree of sexual isolation between intraspecific groups derived from two different gall types. 'Upper type galls' stand on lateral veins of upper leaf surfaces, while 'lower type galls' lie between the lateral veins of lower surfaces. The two groups were not distinguished morphologically but were clearly separated by DNA sequencing. The two gall types coexisted in F. crenata forests and significantly overlapped on host leaves. Hartigiola faggalli is diurnal with one-day adult life span. Emergence, swarming, mating and oviposition occurred successively during particular time slots each day. Each activity was observed almost simultaneously at common sites for the two groups. However, most swarming males flew to females of the same group when responding to female sex pheromone and most mating pairs caught were homogenic. The index of asymmetry (IAPSI) indicated a strong bias to homogenic mating. The female sex pheromone and male sensitivity to the pheromone seemed to differ between the two groups. After mating, females of each group laid eggs correctly on upper or lower surfaces of fresh leaves, respectively. There were a few lower type galls induced on upper leaf surface by the progeny of heterogenic pairs, but sexual isolation apparently existed between the two groups. This may imply that the two groups are possibly at the incipient stage of speciation associated with diversification of pheromone constituents and the development of gall dimorphism.

Keywords:*sexual isolation, Hartigiola faggalli, Fagus crenata, intraspecific groups, gall midge*

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Insect related Interactions at a Multi-trophic Ecosystem S705

Olfactory cues in gall midge speciation

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Gall midges (Diptera: Cecidomyiidae) make ideal models for studying the role of olfaction in speciation: they are speciose compared to other dipterans, short lived and non-feeding as adults (1-2 days), their host plant specificity is legendary and they are strongly driven by olfactory cues. In this study we investigated two types of olfactory cues, the pheromone produced by the female to attract the males as well as plant volatiles used by the female to localize a plant for egg legging. With the crucifer specialist Contarinia nasturtii as our model organism we showed that gall midge host specificity is initiated by the olfactory-based host plant choice of the female. We also demonstrated plasticity in host plant choice, plasticity that can be the basis for the fast speciation in the gall midge family. In addition, the role of gall midge pheromones in speciation is discussed by comparing the structures of the pheromones with the phylogeny of gall midges.

Keywords:*Gall midges, olfaction, speciation, pheromones, host plant volatiles*

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S705F13

Insect related Interactions at a Multi-trophic Ecosystem S705

Electrophysiological screening to investigate gall midge host plant recognition

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Gall midges constitute an excellent model for investigating the role of olfaction in host choice, host shift and speciation. Gall midges have a high rate of speciation and, reportedly, a high degree of specialization concerning host-plant choice. Most of the more than 5000 gall midge (Diptera: Cecidomyiidae) species are plant feeders and many of them are serious pest. The females use the volatiles emitted by plants to locate a host for oviposition. The differentiation of host plants is likely to be based on specific combinations of common plant volatiles that make up unique combinations. As the adults are short-lived (1-2 days), and the larvae have no or limited capacity to disperse, females are under strong selective pressure to find a suitable oviposition site. In an electrophysiological screening (GC-EAD) we investigate the antennal response profile in several gall midge species to a large number of plant compounds. The selection of compounds represents plant volatiles from known gall midge host plants and other compounds known to be involved in plant insect interactions. So far we have successfully tested the synthetic blend on 10 gall midge species, some of them are closely related, some are distantly related species. Whether gall midges associated with the same host plant use the same or similar set of odors? In addition do closely related species that have different host plant respond to odors common for the different plants?

Keywords:*gall midges, Cecidomyiidae, host plant recognition*

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S706F01

Insect related Interactions at a Multi-trophic Ecosystem S706

Ground-dwelling arthropod assemblages following variable retention harvesting: Is green tree retention effective to maintain biodiversity?

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Green tree retention (the practice of leaving standing living trees within harvested cut-blocks) is implemented in different parts of the world to maintain post-harvest legacies similar to those remaining after natural disturbances (e.g., wildfire), and to retain forest structure in harvested areas to improve habitat quality. However, it is unknown how much retention is needed to achieve these conservation goals. The Ecosystem Management Emulating Natural Disturbances (EMEND) project was initiated in 1998 as a large scale (ca. 1000 ha) retention harvest experiment in the boreal forest of western Canada to answer this question and to generally assess the effectiveness of green tree retention in this ecosystem. Thus, EMEND aims to test the short and long term effects of various levels of dispersed tree retention (clear-cut to 75% retention) on biodiversity and to determine which of these practices best maintain assemblages most similar to those in stands that originated from natural disturbances (unharvested controls). Ground-dwelling arthropod assemblages have been collected one, two, five and ten years after harvesting, and we present carabid and spider responses five and ten years post-harvest here. Few differences in assemblages were observed between five and ten years post-harvest. In general, lower retention (10-20%) maintains structural features that are lost after clear-cutting, but higher levels are still needed to retain forest specialists, especially in conifer stands in which there was little evidence of faunal recovery ten years post-harvest. We discuss management and conservation implications of these results.

Keywords:*forest management, green tree retention, spiders and ground beetles, boreal forest, EMEND, Canada*

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S706F02

Insect related Interactions at a Multi-trophic Ecosystem S706

Can intensively managed plantation forests support a diverse arthropod fauna?

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Plantation forests constitute a large proportion of the forest estate in many countries, particularly in the temperate regions of Europe. In addition, large scale deforestation has led to the fragmentation of natural forests within heavily managed agricultural landscapes. In light of this, it is important that the potential of plantations to support a diverse flora and fauna, particularly of forest specialist species, is assessed. We compared arthropod diversity in mixed and single species plantations of non-native conifers to that in native woodlands in forty five stands across Ireland. Spiders and Carabid beetles were sampled with pitfall traps and a range of environmental parameters were measured including vegetation cover, stand structure, soil attributes, and landscape variables such as forest cover. Invertebrate species composition and richness differed between plantations and native woodlands, particularly for key forest-associated species. Responses also differed by taxonomic group. At the stand scale arthropods were related to litter and vegetation cover, whereas large scale variables such as amount of forest cover within 5km were not important. These findings suggest that forest policy aimed at promotion of biodiversity in plantations should support greater diversity of stand structure and tree species composition. The planting of more species of native provenance should also be encouraged.

Keywords:*Spider, Beetle, Plantation forest, Biodiversity*

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S706F03

Insect related Interactions at a Multi-trophic Ecosystem S706

Tropical trees influence trophic structure but not diversity of litter arthropod communities

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Litter arthropods in tropical forests are diverse and patchily distributed in space and time. However, litter arthropods are tied to trees providing food and shelter resources necessary for litter arthropod survival. I review basic theory relevant to this interplay between trees and their litter arthropods. The 'tree' hypothesis posits that environments underneath tree canopies are different from those between canopies in ways that shape arthropod distribution. The 'species' hypothesis posits that different plant species maintain distinct litter environments to which arthropod distribution respond. Because trees varies in the amount of litter they produce, one proposed mechanism for tree or species effects would link greater litter volumes to increased predator/prey ratios by increasing arthropod abundance in lower trophic levels. Besides trophic structure, trees can also affect litter assemblages in other ways, the tree specialization hypothesis proposes that tree-driven habitat heterogeneity maintains litter arthropod diversity. In this talk I present results supporting the tree hypothesis. Trophic structure of litter arthropods responded strongly to litter pools, but are agnostic with respect to the tree species producing it. Furthermore, few links between trees species and arthropod species suggest that reasons other than tree diversity likely promote and maintain litter arthropod diversity in the tropics.

Keywords:*Tropical arthropods, litter depth, predators to prey ratios*

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S706F04

Insect related Interactions at a Multi-trophic Ecosystem S706

Conservation of saproxylic beetles and living tree retention patch size in boreal white spruce forest

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Retention of living trees in harvested landscapes has been strongly promoted as a way to conserve biodiversity, and populations of many saproxylic beetles (i.e. beetles that depend on dead or dying wood during some part of their life cycle) are well known to be threatened by forestry practices. Nonetheless, it remains poorly understood how managing the amount and distribution of residual trees in harvest blocks can best meet conservation goals for saproxylic beetle assemblages. To determine if there is a threshold for retention patch size in spruce forest to minimize loss of saproxylic beetles on harvested landscape, three different scales of retention patches (Small: 0.63-0.94 ha, Medium: 1.59-2.97 ha, Large: 3.34-5.93 ha) were selected. Clear-cut and intact forest areas were also chosen as comparisons. Four window traps were set in each patch (2 on girdled trees and 2 on snags). These were installed in both interior and edge to explore edge effects in these sites. Saproxylic beetles were collected every 3 weeks during the snow-free season of 2010, preserved and identified to species level in the laboratory. Our study will provide crucial information about the role of retention patch size for maintaining diverse saproxylic beetle assemblages in view of edge effect.

Keywords:*Retention patch size, saproxylic beetles, forest management*

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S706F05

Insect related Interactions at a Multi-trophic Ecosystem S706

Soil collembolans of commercial forests plantations of Colombia: Response to plantation age and climatic regime

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This study characterized collembolans diversity and relative abundance in forest commercial plantations of Gmelina arborea and Pachira quinata during the humid and dry periods of 2008 in Zambrano - Bolivar (Colombia). Soil collembolans were sampled in 15 plots using pitfall traps and Berlesse funnel extraction from soil and litter. Specimens were sorted and preserved for further determination to family/genera. Relative abundance was used to compare the effect of plantation age and sampling period using ANOVA. Collembolans from to 3 suborders, 7 families and 14 genera were found adding both climatic periods. The Entomobryidae and the Lepidocyrtus genus predominated in both periods in G. melina plantations. Richness index was expressed as dependent on rain regime and plantation age. On the other hand, in P. quinata the most abundant family was Entomobryidae during humid condition and Bourletiellidae during dry condition. collembolan populations were favored in the oldest plantation during the humid period.

Keywords:*relative abundance, Gmelina arborea, Pachira quinata*

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S706F06

Insect related Interactions at a Multi-trophic Ecosystem S706

Opening the black box: The structure of belowground food webs

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Most of ecological research concentrates on the visible world above the ground. From an ecosystem perspective, however, the belowground system is indispensible as most of the biomass produced by plants enters the decomposer system. Further, the belowground system is closely interconnected to the invisible compartment of plants, roots in soil. The decomposer system recycles dead organic matter thereby forming the basis of plant nutrition. However, decomposer biota not only interact with plants by providing mineral nutrients; rather, soil biota interact in various ways with plant roots via altering their physical, chemical and biological environment. These interactions essentially shape plant performance and, via systemic effects, also the aboveground food web. With the advent of novel technologies, such as stable isotope analysis, fatty acid analysis, molecular gut content analysis and the molecular toolbox for analysing plant performance, interactions below the ground are losing mystery and their role in belowground - aboveground interrelationships is being uncovered. The talk will highlight these novel developments by focusing on trophic interactions in belowground food webs and the interrelationships between decomposer animals, plants and the aboveground food web.

Keywords:*soil food web*

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S706F07

Insect related Interactions at a Multi-trophic Ecosystem S706

Under which conditions does retention harvesting support boreal ground-dwelling forest arthropods?

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Forestry has impacted North European forests for hundreds of years, but a considerably more intense era begun only some 50 years ago. This intensification has speeded up the rate of extinctions among forest species. Indeed, in Fennoscandia, forestry is considered one reason for the decline of one-third of threatened species. This finding has recently forced forest industries to partly replace clear-cut harvesting with other logging methods, and to change stand structures toward greater heterogeneity in tree composition. Here, I will review the ecological impacts of these actions, with special attention to ground-dwelling arthropods. Responses of arthropods to logging are variable and depend e.g. on species' habitat affinities. At the stand level, retention of 60-70% of the total tree volume will support most ground-dwelling arthropods. The role of structural heterogeneity in supporting forest-arthropod assemblages in managed forests is poorly understood, but recent studies suggest this effect to be significant.

Keywords:*Spider, Beetle, Ant, Green tree retention, Key biotope*

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Substrate diversity promotes species diversity in saproxylic beetles

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Saproxylic (wood living) invertebrates are a major component of forest biodiversity and ecosystem function. At the same time many saproxylic species are disfavoured by forestry. Considerable effort is therefore made to maintain diversity of these species. However, our knowledge on substrate requirement of these species is limited which decreases conservation efficiency. We conducted a large-scale field experiment was to evaluate the relative importance of manipulated microhabitats, i.e. dead wood substrates of spruce (snags, and logs that were burned, inoculated with wood fungi or shaded) for species richness, abundance and assemblage composition of all saproxylic and red-listed saproxylic beetles, separately. Beetles were collected in emergence traps in 30 forest stands. Assemblages of saproxylic beetles differed significantly between substrate types, most pronouncedly between tree species (birch and spruce) and between standing and lying dead wood. Rarefaction curves showed that species richness increased significantly faster with increased substrate diversity. Our results clearly stress the importance of substrate diversity for maintaining saproxylic diversity. It also suggests that some types of variation in substrate characteristics are more important than other but this also depends on which forest type they are placed in. The implication of our results for practical biodiversity management is discussed.

Keywords:*Dead wood, beetles, assemblage composition, biodiversity, conservation*

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Insect related Interactions at a Multi-trophic Ecosystem S706

Survey on distribution and species diversity of oak gall wasps (Hymenoptera: Cynipidae) species in West-Azerbaijan, Iran

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The objective of this study was to determine oak gall wasps diversity in six different locations. So, in this survey, the oak gall wasps were collected from oak forests of West Azerbaijan province during growth seasons (from April to November 2010 and 2011). The galls occurred on oak trees were collected from Pardanan, Mirabad, Nalas, Kavlan, Hamran and Sardasht locations. The number of galls on sampled branches which were 50 cm in diameter and from four cardinal directions on each tree was counted. Species richness of oak gall wasps was estimated and also parameters such as Simpson's index, Shannon's H', and Sorensen similarity quotient were calculated. In this survey, 8 and 30 species of oak gall wasps on oak trees (Quercus infectoria, Q. brantii and Q. libani) were identified in the spring and fall, respectively. The most amount of galls occurred on Q. infectoria. All of the collected gall wasps belong to seven following genera: Andricus, Cynips, Neuroterus, Chilaspis, Pseudoneuroterus, Biorhiza and Aphelonyx. Among these oak gall wasps, 25 species were related to the Andricus genus. The highest amount of Simpson (0.8) and Shannon (2.1) indices were recorded in Pardanan area and also the highest evenness was recorded in Kavlan (0/9). The highest amount of Sorensen similarity was recorded between Pardanan and Sardasht (85 %). The most important point is that the role of oak subspecies and climate of regions should not be ignored in distribution of oak gall wasps.

Keywords:*Diversity, Gall, Wasp, Distribution, Similarity*

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S706F10

Insect related Interactions at a Multi-trophic Ecosystem S706

Changing climate associated with homogenization of forest assemblages of boreal ground beetles (Coleoptera: Carabidae)

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Carabid assemblages were studied in 1981-82 and 2009-10 using pitfall traps in five habitats in central Alberta, Canada. Traps were run overnight weekly without killing agents during the frost-free season in all years. Overall average catch rate was 39.2% lower in the later period than during 1981-82 (1.62 vs 0.65 beetles/trap/day). Carabid activity was reduced in Lakeside Forest (LS) and Meadow (ME) habitats, but higher in Spruce Forest (SF). Shannon-Weiner diversity (H') (2.73 vs. 2.45) fell between the two study periods, as did overall species numbers (56 vs 34). The 1981-82 catch included 32 species not subsequently collected; 21 (15 only once and 6 less then 5) were relatively rare (≤ 5 specimens), but many had been common earlier, including Pelophila rudis, Elaphrus clairvillei and Platynus mannerheimii. Ten species captured during 2009-10, had not been detected during the earlier sampling period. These included Synuchus impuctatus, Metabletus americanus and the invasive exotic species, Pterostichus melanarius. A Multiple Regression Tree analysis explained 84.4% of variation in the assemblages and identified differences between ME and the four forest habitats, the two sampling periods, and LS vs. SF, Upland Aspen (UA) and Moist Aspen (MA) forests as explaining, respectively, most variation in the assemblages. A Redundancy Analysis suggests that forest carabid assemblages have become more homogeneous in the later years. Weather records from nearby indicate that climate has become warmer and drier during the study period and our data support the hypothesis that climate effects are associated with changes in this local carabid fauna.

Keywords:*Carabidae, community structure, climate change, habitat use*

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S801M01

Pesticides, GM Crops, Resistance and Toxicology

Juvenile hormone biosynthetic enzymes as targets for insecticide discovery

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Juvenile hormone (JH) plays important roles in insect morphogenesis and reproduction. For this reason, interfering with its biosynthesis has long been considered a promising strategy for the development of target-specific insecticides. Although several inhibitors of JH biosynthetic enzymes have been developed, none have yet been used operationally for the management of insect pests. However, recent progress in the fields of molecular biology and structural bioinformatics has made it possible to clone and characterize key JH biosynthetic enzymes, opening the way to the rational design of highly specific and effective enzyme inhibitors. In addition, the discovery of insect-produced peptides that inhibit JH biosynthesis, the allatostatins, has led to the development of peptide analogs that inhibit JH biosynthesis in vivo, offering an alternative approach for interfering with JH production. This presentation will provide an overview of recent work aimed at the cloning and characterization of JH biosynthetic enzymes and the development of inhibitors of JH biosynthesis.

Keywords:*Juvenile hormone biosynthesis, mevalonate pathway, juvenile hormone-specific pathway, enzyme inhibitors, allatostatin analogs*

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S801M02

Pesticides, GM Crops, Resistance and Toxicology

Plant secondary compounds from *Leucaena leucocephala, Alpinia zerumbet* and *Bidens pilosa*

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The search for botanical insecticides has increased recently due to the adverse effects of synthetic insecticides and the eco-toxicological properties. Risks and problems associated with the use of chemicals lead to increasingly stringent environmental regulation of pesticides, and hence, the need of developing safer and eco-friendly alternatives that have potentiality to replace synthetic insecticides is imperative. The plants Alpinia zerumbet, Leucaena luecocephala and Bidens pilosa are extensively found in Okinawan islands. Several compounds from Alpinia spp. have been identified to have insecticidal activities against Drosophila, Spodoptera, Sitophilus, Tribolium, etc. We have previously isolated dihyro-5,6-dehydrokawain (DDK) from alpinia and showed the insecticidal activity of DDK-derivatives against Coptotermes formosanus. Furthermore, the plant Leucaena contains a non-proteinogenic amino acid, mimosine, which have been reported to possess insecticidal activity. Besides, proteinase inhibitors from Leucaena have also been identified as a bio-insecticide. Studies regarding utilization of B. pilosa as a natural source of insecticides are very scarce, however, several studies on this plant has clearly indicated its possibility to be a bio-insecticide. Therefore, further researches in these plants regarding the development of natural insecticides are very imperative. Since our laboratory always focuses on total utilization of Okinawan plants, we are continuing our research in, firstly, identifying the potent natural insecticides from these plants and secondly, synthesizing derivatives of these natural compounds to develop more potent, yet safer, insecticides.

Keywords:*Leucaena leucocephala, Alpinia zerumbet, Bidens pilosa, mimosine, dihydro-5,6-dehydrokawain*

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Design of molting hormone agonists

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20-Hydroxyecdsysone (20E) regulates the insect molting, and the disruption of the action of 20E leads insects to death. It has been thought that 20E-like compounds have been thought to be insect growth regulators (IGRs), and diacylhydrazine (DAH)-type compounds came into the pesticide field two decades ago. To date, five DAHs (tebufenozide, methoxyfenozide, chromafenozide, halofenozide, and fefenozide) are commercialized as insecticides, but these DAHa besides halofenozide are specific to Lepidoptera. This is due to the fact that the binding site of DAH is slightly different from that of 20-hydroxyecdysone that is commonly used as a molting hormone in insect and other Ecdysozoa. Therefore, the compounds that bind to the 20E-binding site of EcR are able to be IGRs with broad insecticidal spectrum. We recently performed the ligand-based virtual (in silico) screening to find novel ecdysone agonists using the OpenEye software and in-house program. As the template ligand molecule ponasterone A (PoA) that is more potent than 20E was used. Three million compounds were screened down to 20,000 compounds, then to 237 compounds based on QSARs derived for PoA analogs. Finally 24 compounds were submitted to the cell-based binding assay using Sf-9 and BCIRL-Lepd-SL1 cells. Three novel compounds specifically bound to the molting hormone receptor of both cell lines but did not show the agonistic activity at 100 μM. It was demonstrated that these compounds reasonably bound to the ligand binding domain of the molting hormone receptor for Sf-9, which is modeled from the crystal structure of Heliothis virescence EcR.

Keywords:*Ecdysone receptor, 20-hydroxyecdysone, insect cells, QSAR, diacylhydrazine*

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Ligand-gated chloride channels: Important sites for developing novel insecticides

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The nervous system function is maintained by the balance of the cell-to-cell communication mediated by the excitatory and inhibitory neurotransmitters at synapses. In insects, acetylcholine plays an important role as an excitatory transmitter, while γ-aminobutyrate (GABA) serves as a major inhibitory transmitter. Glutamate functions as both excitatory and inhibitory transmitters in insects. Excitatory transmitters open cation channels for transmembrane signaling, whereas inhibitory transmitters open anion (chloride) channels. Inhibition or excessive stimulation of these channels causes lethality in insects. Therefore, these channels are critical targets for insecticides and parasiticides. The first generation of insecticides that target the chloride channels include γ-BHC and cyclodienes, which act as negative allosteric modulators (NAMs), and it can be said that the second generation insecticides are phenylpyrazoles with a similar NAM activity. Recently, we reported that the antiparasitic isoxazoline A1443 uniquely blocks GABA- and glutamate-gated chloride channels (GluCls) with selectivity to the former channel. A1443 acts at a site distinct from the site for the first and second generations of NAM insecticides. A1443 can be categorized as the third generation of NAMs that target GABA-gated chloride channels. In contrast to NAMs, insecticidal and antiparasitic macrolides activate GluCls as positive allosteric modulators. These macrolides have unique site(s) in GluCls. Another important ligand binding site is the orthosteric site, which is the site for agonists and competitive antagonists. Design and synthesis of chemicals that fit into these sites will lead to the development of the next generation of pest control chemicals.

Keywords:*GABA, glutamate, receptor, channel, insecticide*

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S802M01

Pesticides, GM Crops, Resistance and Toxicology

Genetic mapping of etoxazole resistance mutations in *Tetranychus urticae* provides new insights in the mode of action of chitin synthesis inhibitors

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We developed a population, multi-generational bulk segregant analysis (BSA) mapping method to identify etoxazole target-site resistance mutations and thus the molecular target for the acaricide etoxazole in the spider mite, Tetranychus urticae, an important global pest. Etoxazole represents one of several compound classes that act as potent chitin biosynthesis inhibitors, and that are of great agricultural importance for the control of arthropod populations. We identified a nonsynonymous variant in the major T. urticae chitin synthase enzyme that confers target-site resistance to etoxazole, suggesting a direct interaction between etoxazole and chitin synthase. Identification of the target-site of a chitin synthase inhibitor is important to fully exploit chemical structures. Intriguingly, the nature of the CHS1 mutation underlying resistance is potentially informative as to why the exact mode of action of arthropod chitin biosynthesis inhibitors have remained elusive for decades: the target site mutation suggests that inhibition acts on a non-catalytic (but essential) function of CHS1 complexes.

Keywords:*genetic mapping, chitin synthesis, etoxazole*

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Frequencies of the M918I, T929I and L1014F mutations in the sodium channel of the diamondback moth in China, Thailand and Japan

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In addition to the allele frequencies of the L1014F and T929I mutations which are involved in nerve-insensitive resistance to a pyrethroid in the diamondback moth, those of the M918I mutation were examined using field strains obtained in China, Thailand, and Japan. Results show that the resistance allele frequencies at the L1014F site were 89%-100%, 97%-100% and 65%-100%, respectively, for strains in China, Thailand, and Japan. The respective allele frequencies at the T929I site were 86%-100%, 70%-97% and 58%-84% for Chinese, Thai, and Japanese strains. With low frequencies up to 27%, M918I was found in Japan and China, but not in Thailand. The strain homozygous for the M918I and L1014F mutations was established and its resistance level to a pyrethroid was examined. The strain lacks a portion of the sodium channel gene corresponding to the 3´ portion of exon 18a, intron 18, and the 5´ portion of exon 18b. Nevertheless, the strain showed a similar level of resistance to that which was homozygous for the T929I and L1014F mutations.

Keywords:*Diamondback moth, Insecticide resistance, Plutella xylostella, Pyrethroid, Sodium channel*

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S802M03

Pesticides, GM Crops, Resistance and Toxicology

Resistance to diamide insecticides in the diamondback moth in China

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Chlorantraniliprole is the first commercial insecticide from a new class of chemistry, the anthranilic diamides. Chlorantraniliprole provides an effective alternative insecticide for control of Plutella xylostella populations resistant to other insecticides. Baseline susceptibility to chlorantraniliprole for P. xylostella was surveyed from 16 geographical populations sampled from China during 2008-2009, and the median lethal concentrations (LC50s) varied among populations from 1.8- to 8.9-fold higher than the LC50 of a susceptible strain (Roth). Twenty field populations of P. xylostella sampled in 2010-2011 from China were tested with laboratory bioassays to determine if resistance to chlorantraniliprole had evolved in the field. The LC50s of the 14 populations from northern China ranged from 1.7- to 5.4-fold compared with the LC50 of Roth, which indicates these populations remain susceptible to chlorantraniliprole. However, the LC50s of the 6 populations from southern China (Guangdong Province) were 2.6-, 12-, 18-, 81-, 140-, and 2,000-fold higher than the LC50 of Roth. The results showed that high levels of resistance to chlorantraniliprole had evolved in field populations from Guangdong Province of southern China. Cross resistance, inheritance mode and resistance mechanisms of the field-evolved resistance to chlorantraniliprole were preliminarily characterized. Intensive use and misuse of chlorantraniliprole may be responsible for the rapid evolution of high-level resistance in P. xylostella from southern China. The implementation of resistance monitoring plans and resistance management strategies is urgently needed in China to preserve susceptibility to chlorantraniliprole in P. xylostella.

Keywords:*Plutella xylostella, chlorantraniliprole, resistance*

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S802M04

Pesticides, GM Crops, Resistance and Toxicology

Dynamics of insecticide resistance in the peach-potato aphid, *Myzus persicae*

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In the UK and elsewhere in northern Europe, Myzus persicae is largely anholocyclic, consisting of relative few clonal lineages each with a characteristic and constant suite of resistance genotypes. As a result, the dynamics of resistance genes must reflect the outcome of inter-clonal selection across the genome as a whole as much as selection acting at individual loci. The congealing and persistence of such multi-locus and co-adapted genotypes within clones has interesting and important implications for tactics aimed at combating established and new resistance mechanisms. On the one hand, it should buffer resistance genes from the temporal and spatial fluctuations in frequency that reflect the interplay of fitness costs and insecticide use and which often occur in sexually-outcrossing organisms. More controversially, it could also buffer against the rapid invasion of novel resistance genes, whose success depends not only on their appearance in a new area but also on their rapid incorporation through recombination into locally-adapted gene pools. These ideas will be discussed with reference to long-term aphid resistance monitoring data from the UK and a prognosis for the northwards spread of a novel target-site based mechanism of resistance to neonicotinoid insecticides that is currently confined to southern Europe.

Keywords:*insecticide resistance, aphid pest management, population genetics*

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Mechanisms of resistance to neonicotinoid insecticides in the peach-potato aphid, *Myzus persicae*

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Myzus persicae is a serious pest on crops all over the world causing damage through feeding and pathogen transmission. It has a long history of developing resistance to most insecticides used for its control. The neonicotinoids, an important class of insecticides that target specific receptors in the insect's nervous system have been used effectively to control M. persicae for over two decades. However, intensive reliance on these compounds has led to a gradual increase in resistance, culminating with the loss of field effectiveness in some fruit orchards in southern Europe. Research into the mechanisms underlying resistance to neonicotinoids in M. persicae showed that cytochrome P450-mediated detoxification together with possible additional mechanisms like slower cuticular penetration account for low levels of resistance that do not compromise field control. However, the most potent resistance is conferred by a point mutation in the nicotinic acetylcholine receptor (nAChR) - the neonicotinoid target site. Evidence for the proposed mechanisms of resistance will be presented in selected M. persicae clones and the effect of the identified target-site mutation discussed in the context of previous studies on neonicotinoid insecticide selectivity for the insect nAChR.

Keywords:*Myzus persicae, neonicotinoid insecticides, nicotinic acetylcholine receptor, cytochrome P450, target-site resistance, metabolic resistance*

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Neonicotinoid resistant *Myzus persicae* demonstrate an increased ability to locate untreated plant tissue compared to a susceptible clone

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The peach potato aphid Myzus persicae is a major agricultural pest with the ability to transmit over 100 plant viruses to a wide range of crops. Control relies greatly upon treatment with neonicotinoid insecticides e.g. thiamethoxam. In 2009 the FRC clone was discovered on peach in France. This clone exhibits between 255-1679 fold resistance to current neonicotinoids previously linked to metabolic and target site resistance. It is possible that dispersal behaviour may enhance the resistance of an insect by causing it to remain upon treated plants for a shorter length of time and so ingest a lower dose of insecticide. This study investigated if this was the case with the FRC clone, and is the first to compare the dispersal behaviour of aphid clones of the same species with differing levels of resistance to insecticides. The dispersal behaviour of the FRC clone was compared with that of a clone of lower neonicotinoid resistance, 5191A, and the susceptible clone US1L. The data highlighted several differences in dispersal behaviour between these clones. Most importantly, the FRC clone exhibited an increased ability to locate untreated areas when presented with an environment consisting of both thiamethoxam-treated and untreated plant tissue. This behaviour was not seen in the other clones, suggesting that altered dispersal behaviour may partially account for the high level of neonicotinoid resistance exhibited by the FRC clone. Since the dispersal of aphid vectors is key to the transmission of viruses across crop fields, this has implications for current crop protection practice.

Keywords:*Aphid, Behaviour, Resistance, Dispersal, Neonicotinoid*

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S802M07

Pesticides, GM Crops, Resistance and Toxicology S802

Activities of the Insecticide Resistance Action Committee (IRAC), with particular emphasis on Asia

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The Insecticide Resistance Action Committee (IRAC), formed in 1984 and a subgroup of CropLife International, currently consists of 14 member companies and is committed to ensuring the sustained effectiveness of the industry's products. The mission of IRAC is thus to facilitate communication and education on insecticide resistance and to promote the development of resistance management strategies in crop protection and non-agricultural insecticide uses to support sustainable agriculture, improved public health and enhanced professional product use. IRAC has a role in initiating key research and extension projects as well as seeking to improve the measurement and understanding of resistance development. In addition, IRAC International supports IRAC country groups to devise and promote effective local solutions to insecticide resistance problems. IRAC maintains and promotes the global classification of modes of insecticide action, and using this information it is possible to construct appropriate IRM strategies using a range of available products. Depending on the insect, it is possible to use insecticides which act at alternative target sites or which are resisted in different ways, thus maintaining the efficacy of the various insecticidal components of a crop protection strategy. The overall aim of this effort is to keep all classes of insecticides and acaricides as viable control options and to protect the long-term viability of insecticide use.

Keywords:*Insecticide Resistance Action Committee, IRAC*

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Identification of imidacloprid resistance mechanism in the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae)

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The cotton aphid, Aphis gossypii (Glover), is also one of the most serious pest in various vegetables. Neonicotinoid insecticides exhibit niceish insecticidal activity against Myzus persicae in Korea. However, some field populations of A. gossypii showed high resistant against neonicotinoid. The imidacloprid-resistant strain (IR) selected from that populations and that was 278 folds more resistant to imidacloprid compared to a susceptible strain (S) as judged by LC50 values. The IR showed cross resistances to other neonicotinoid insecticides. To identify differentially expressed genes in IR, comparative transcriptome analyses based on GS-FLX were conducted using total RNAs extracted from both IR and S strains. Seven ATP-binding cassette (ABC) transporter genes were newly identified in A. gossypii, among which only ABCC9 gene was highly expressed in IR. Therefore, this ABCC subfamily, a member of the MRP subfamily which is involved in multi-drug resistance, could be one of the factors associated imidacloprid resistance in A. gossypii. Comparison of the nucleotide sequence of six nicotinic acetylcholine receptor (nAChR) subunit (alpha 1-5, beta 1) genes from S and IR strain revealed a point mutation in the loop D region of the nAChR beta 1 subunit of the IR, causing an arginine to threonine substitution (R81T). These mechanisms also reported in M. persicae and this amino acid change confers a vertebrate-like character to the insect nAChR and results in reduced sensitivity to neonicotinoids. This is the first investigation of imidacloprid resistance mechanism in A. gossypii and also can apply to manage A. gossypii resistant populations in field.

Keywords:*Aphis gossypii, insecticide resistance, next generation sequencing, ATP-binding cassette transporter*

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Resistance situation against neonicotinoid insecticides in the brown planthopper, *Nilaparvata lugens*

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Insecticide resistance against imidacloprid has developed in the brown planthopper Nilaparvata lugens (Stal) in many Asian countries since 2005. The susceptibility of BPH against five neonicotinoid insecticides (imidacloprid, thiamethoxam, dinotefuran, clothianidin, and nitenpyram) were monitored by topical application method from 2006 to 2010 in East Asia (Japan, China, and Taiwan), Northern Vietnam (the source area of the BPH migration to East Asia), and Southern Vietnam (Mekong delta). The LD50 values of BPH against imidacloprid increased from 2006 to 2009, and have slightly decreased in 2010 in all the areas tested. The peak values of the LD50's were 80μg/g both in East Asia and Northern Vietnam. The patterns of annual change in the LD50 values against imidacloprid was quite similar between East Asia and Northern Vietnam, suggesting that the resistance situation in East Asia to which the planthoppers immigrated reflects the situation in the source area (Northern Vietnam). In contrast, the LD50 values in Southern Vietnam were 150-250μg/g, suggesting that BPH resistance to the imidacloprid is on the rise in the Mekong delta. In the Mekong delta, the LD50 values of thiamethoxam and clothianidin were 10μg/g at a maximum, but those in dinotefuran and nitenpyram were 2μg/g. A significant correlation was found in the LD50 values between imidacloprid and the other four neonicotinoids. Speamann's rank correlation coefficient was high in the two combinations, i.e., imidacloprid v.s. thiamethoxam and imidacloprid v.s. clothianidin.

Keywords:*insecticide resistance, neonicotinoid, imidacloiprid, topical application*

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S802M10

Pesticides, GM Crops, Resistance and Toxicology

Neonicotinoid and spinosad resistance in *Musca domestica*; from bioassay to transcriptome

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The molecular mechanisms responsible for resistance are important in responce to alterations in susceptibility in insect pest populations and to develop strategies to prevent development of resistance. Neonicotinoids and spinosad were introduced for insect control within the last decade and play in many countries an essential role for the control of house flies and other insects. We have identified neonicotinoid- and spinosad-resistant housefly strains questioning the future use of these insecticides targeting the neural nicotinic acetylcholine receptor. Females of a multi-resistant field population were spinosad-resistant, whereas male house flies of this strain were susceptible. Selection of this population with two neonicotinoids, spinosad and fipronil led to four strains with an interesting pattern of cross-resistance. We present differences in expression of nACh-receptor subunit Mdα2 as well as gender specific differences between expression of CYP6D3, CYP6A1, CYP6D1, CYP6G4, CYP6A36, CYP6A37, CYP12A1 and CYP12A2 and elucidate their response to sublethal doses of spinosad and phenobarbital. Comparison of transcriptome (400.000 sequences averaging 250 bp) from insecticide-resistant and susceptible house fly strains revealed interesting difference CYP6D1 from resistant and susceptible transcriptome.

Keywords:*Musca, neonicotinoid, cytochrome P450, transcriptome*

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S802M11

Pesticides, GM Crops, Resistance and Toxicology

Dynamics and mechanisms of insecticide resistance in European populations of the pollen beetle, *Meligethes aeneus*

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Pollen beetle, Meligethes aeneus F. (Coleoptera: Nitidulidae) is a major pest in European oilseed rape. Winter oilseed rape is cultivated on several million hectares in Europe. The continuous use of pyrethroids resulted in high selection pressure and subsequent development of resistance. Pyrethroid resistance in pollen beetle is now widespread in many European countries. A resistance monitoring using an adult vial test revealed high levels of resistance to numerous pyrethroids. Detailed cross-resistance and synergism studies were carried out using the same assay design. Synergism experiments revealed a high synergistic potential of piperonyl butoxide in vivo. A PCR approach with degenerate primers for conserved regions of CYP6 family revealed 13 CYP-like sequences from pollen beetle cDNA pools. Evaluation of expression levels by RT-qPCR showed that one gene (CYP6BQ23) is highly overexpressed (>200 fold) in resistant populations. Recombinant expression of CYP6BQ23 using a baculovirus expression system with a Spodoptera frugiperda cell line confirmed a significant role of this CYP for pyrethroid resistance, and the forming of 4'OH-deltamethrin. Elevated expression of CYP6BQ23 is correlated with resistance levels in pyrethroid-resistant populations throughout Europe. In contrast a second mechanism, i.e. target-site resistance conferred by an L1014F mutation in the para-orthologous voltage-gated sodium channel known as kdr (knock down resistance) seems to be restricted to populations from northern Europe, especially Denmark and Sweden. For further investigations of cross-resistance patterns and the characterization of the pyrethroid target site the coding sequence of the para-orthologous gene was isolated.

Keywords:*Pyrethroid resistance, kdr, Cytochrome P450 monooxygenases*

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A nicotinic acetylcholine receptor point mutation associated with resistance to spinosad in western flower thrips (*Frankliniella occidentalis*)

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High levels of resistance to spinosad, a macrocyclic lactone insecticide, have been reported previously in western flower thrips, Frankliniella occidentalis, an economically important insect pest of vegetables, fruit and ornamental crops. We have cloned the nicotinic acetylcholine receptor (nAChR) α6 subunit from F. occidentalis (Foα6) and compared the nucleotide sequence of Foα6 from susceptible and spinosad-resistant insect populations (MLFOM and R1S, respectively). A single nucleotide change has been identified in Foα6, resulting in the replacement of an amino acid in the transmembrane domain of Foα6. Whilst there is no direct evidence identifying the location of the spinosad binding site, the analogous amino acid in the C. elegans glutamate-gated chloride channel lies in close proximity to the known binding site of ivermectin, another macrocyclic lactone pesticide. The functional consequences of the resistance-associated mutation have been examined in the human nAChR α7 subunit. Introduction of an analogous mutation in α7 abolishes the modulatory effects of spinosad whilst having no significant effect upon activation by acetylcholine. In addition, in radioligand competition binding studies, spinosad did not displace binding of bungarotoxin to the α7 nAChR. These data are consistent with spinosad having an allosteric mechanism of action.

Keywords:*Spinosad*

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S803TU01

Pesticides, GM Crops, Resistance and Toxicology

Risk assessment of genetically-modified maize debris on stream-inhabiting arthropods

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We assessed risk of Bt maize debris on stream arthropods based on exposure and non-target effects, with a focus on the movement of senesced crop debris into streams. For exposure, we measured debris movement into streams and the persistence of Bt bioactivity with time. For effect, we evaluated Bt and non-Bt near-isoline senesced leaf tissue on aquatic arthropods. In addition, we measured decomposition and colonization rates of leaf tissue in streams. From our results on maize modified with the Cry1Ab gene, we found rapid loss of Bt bioactivity with environmental exposure as well as a delay in the movement of leaf debris into streams, suggesting a lack of significant exposure of Bt to stream organisms. Our effect studies demonstrated no harm to trichopteran leaf-shredding species, while a tipulid crane fly and an isopod exhibited reduced performance on one Bt variety. In addition, decomposition and colonization rates varied among streams, but were not related to Bt. Our results provide evidence that adverse effects to aquatic non-target shredders involve complex interactions arising from plant genetics and environment which cannot be ascribed to the presence of Cry1Ab proteins. More recently, our results with stacked varieties show significantly increased persistence of Bt in maize tissue, extending exposure for several months after harvest. Thus, the balance of exposure and non-target effects in risk analysis may be altered with the deployment of new varieties, placing more value on tests of the potential non-target effect of Bt maize on stream invertebrates.

Keywords:*stream insects, Bacillus thuringiensis, Trichoptera*

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Genetically modified crops and aquatic ecosystems: Considerations for environmental risk assessment and non-target organism testing

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Environmental risk assessments (ERA) support regulatory decisions for the commercial cultivation of genetically modified (GM) crops. The ERA for terrestrial agroecosystems is well-developed, whereas guidance for ERA of GM crops in aquatic ecosystems is not as well-defined. The purpose of this document is to demonstrate how comprehensive problem formulation can be used to develop a conceptual model and to identify potential exposure pathways, using Bacillus thuringiensis (Bt) maize as a case study. Within problem formulation, the insecticidal trait, the crop, the receiving environment, and protection goals were characterized, and a conceptual model was developed to identify routes through which aquatic organisms may be exposed to insecticidal proteins in maize tissue. Following a tiered approach for exposure assessment, worst-case exposures were estimated using standardized models, and factors mitigating exposure were described. Based on exposure estimates, shredders were identified as the functional group most likely to be exposed to insecticidal proteins. However, even using worst-case assumptions, the exposure of shredders to Bt maize was low and studies supporting the current risk assessments were deemed adequate. Determining if early tier toxicity studies are necessary to inform the risk assessment for a specific GM crop should be done on a case-by-case basis, and should be guided by thorough problem formulation and exposure assessment. The processes used to develop the Bt maize case study are intended to serve as a model for performing risk assessments on future traits and crops.

Keywords:*Environmental risk assessment, aquatic ecosystem, non-target organism, genetically modified crops*

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Non-target effects of GM crops: A global synthesis

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Despite the wide scale cultivation of GM crops globally and the large number of non-target studies that have been completed over the past 15-20 years in support of risk assessment, there remain concerns about the unexpected effects of the technology on the environment. A number of reviews and several meta-analyses have been conducted in an attempt to summarize and synthesize the extant literature. In general, these summaries and analyses have demonstrated a general lack of effect on non-target abundance in the field and on various measures of their biology in the laboratory. However, many additional studies have been published since these synthetic studies were completed and additional new guidance has become available that help to better assess the veracity of all non-target studies currently available in the literature. Here we will re-assess the world literature and quantify non-target effects through meta-analyses. Attention will be placed on non-target abundance and diversity in the field and on life history in the laboratory. Special focus will be placed on natural enemies important in biological control by examining effects on abundance, biology and biological control function. Finally, we will further examine the connection between laboratory and field results in order to inform the tier-testing system used by many regulatory authorities.

Keywords:*risk assessment, biological control, meta-analysis, non-target effects, GM crops*

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Evaluation of Genuity® Bollgard III® cotton effects on non-target organisms

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The Australian cotton industry has almost universally adopted Bt-transgenic cotton varieties (currently Bollgard II® expressing Cry1Ac and Cry2Ab) primarily due to their effective suppression of target Helicoverpa pests. Resistance development in Helicoverpa was a recognised threat prior to the release of these technologies, and a pre-emptive resistance management plan developed and implemented. To further mitigate the risk of resistance in Helicoverpa increasing, Monsanto has developed Genuity® Bollgard III®, which expresses an additional insecticidal protein (VIP3A). In transgenic cotton varieties, VIP3A alone demonstrates relatively few effects on non-target organisms (Whitehouse et al. 2007). Extensive worldwide research on Cry proteins has demonstrated limited to no direct or indirect toxicity to non-target organisms. Likewise, assessments within Bt-crops at the community level demonstrate few consistent non-target effects. However, the combined expression of VIP3A, Cry1Ac and Cry2Ab in Bollgard III® may pose a risk to non-target organisms. Here we present the latest findings from field assessments of Bollgard III® non-target effects in Australia. To date, results demonstrate that the arthropod assemblage associated with Bollgard III® is not significantly different to that of Bollgard II®. Significant seasonal divergence between arthropod assemblages associated with Bollgard III® and non-Bt cotton is largely driven by increasingly disparate pest larval densities. At present, we believe it is unlikely that Bollgard III® will affect non-target organisms in a different manner to currently utilized Bt-transgenic varieties. Evidence suggests the commercial release of Bollgard III® would help ensure the ongoing effectiveness of Bt-transgenics on which the Australian cotton industry is almost exclusively reliant.

Keywords:*Bollgard III, Bt-transgenic cotton, Arthropod assemblage, Non-target organisms*

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Bioassay of cry1 ab protein to non-target Lepidoptera, the great egg fly, *Hypolimnas bolina philippensis* Butler (Nyphalidae : Lepidoptera)

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Bioassay using the maximum hazard dose (i.e. approximately 10x the maximum Cry1Ab protein concentration in Bt corn) was done by feeding three-day old larvae of the Great Egg Fly butterfly, Hypolimnas bolina philippensis Butler with the natural diet of Ipomoea triloba L. treated with Cry1Ab protein. Leaf discs (2 cm diameter) were soaked in Cry1Ab solution and air dried. One larva was placed inside the plastic cup containing the Cry1Ab-treated leaf disc. The larvae were transferred on leaves without Cry1Ab after 24 hours. For control assay, the leaf was soaked in carbonate buffer. Fifty larvae were used per treatment with a total of nine treatments for each group. Larval mortality and weight were recorded after seven days. T-test was used to analyze the data. Another test on intact leaves of Ipomoea batatas (L.) was prepared. The larvae were placed in air dried leaves soaked in Cry1Ab protein. The treated leaf with twenty five larvae was put inside a black cylindrical board cage to protect the Cry1Ab protein from the degrading effect of light. The intact leaf was used to prevent secondary metabolites effect on the larvae. The same number of larvae and treatments were utilized. The mean mortality (0.632) and mean weight (-0.404) differences on leaf disc method of the treated and control leaves were not significant. Conversely, the mean mortality (-0.0191) and mean weight (-0.957) differences on intact leaves of the two groups were not significant. The findings indicate that the Cry1Ab protein was not toxic to H. bolina.

Keywords:*Bioassay, Cry1 Ab protein, Bt corn, Hypolimnas bolina, Mortality, Larval weight, T-test*

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A comparison of canopy-dwelling non-target arthropod communities in open pollinated Bt and non-Bt eggplant in Three multi-location trial sites in Luzon Island, Philippines

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Canopy-dwelling non-target arthropods present in open pollinated Bt and non-Bt eggplant varieties were monitored in three multi-location trial sites during two cropping seasons in 2010-2011. Visual count of arthropods was done at weekly interval for fourteen weeks. Redundancy analysis (RDA) of abundance data and Principal Response Curve (PRC) analysis of repeated measures to test and display treatment effects that change across time were done using CANOCO for Windows v4.5. Significance was tested using the unrestricted Monte-Carlo method (499 permutations under reduced model). Biplot diagrams of the ordination results from redundancy analysis of abundance data from each site over two seasons were generated using CanoDraw for Windows v4.13. RDA analysis showed that leafhopper, Amrasca biguttula (Ishida)and whitefly, Bemisia tabaci (Gennadius) were the highly dominant species while only two species, lepidopteran leafminer, Phycita clientella Zeller and semilooper, Chrysodeixis eriosoma, (Doubleday) were strongly associated with non-Bt eggplant. However, no significant differences in arthropod communities between Bt and nonBt eggplant varieties across sites over two seasons were detected by PRC. Variance explained by crop type range from 4.2 to 8.5% except at 26.0% in one season for UPLB site while variance explained by sampling period range from 23.4-56.3%.

Keywords:*BT eggplant, multi-location trial, non-target organism, arthropod communities*

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Effects of GM crops on nontarget organisms: Field studies in India

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Deployment of Bt-transgenic cotton has led to significant reduction in pesticide usage and health hazard, enhanced environmental quality, increased crop production, and improved the efficacy of other pest management tactics. However, the concerns raised regarding effects of large-scale cultivation of Bt-cotton on the nontarget organisms, arthropod biodiversity, development of resistance, and pest resurgence are genuine. Monitoring of arthropods in farmers fields in India indicated that the populations of major nontarget sucking insect pests viz., Amrasca biguttula biguttula, Bemisia tabaci, Aphis gossypii, Oxycarenus laetus, Dysdercus koenigii, and Nezara viridula; and the generalist predators viz., Cheilomenes sexmaculatus, Chrysopa spp., and spiders did not differ significantly between Bt and non-Bt cottons. Species richness and diversity index of the plant inhabiting and soil dwelling arthropods were similar in Bt and non-Bt cottons. However, the reduction in insecticide sprays, especially during flowering and boll formation phases, has resulted in resurgence of some minor pests such as mealy bugs, Spodoptera litura, thrips, aphids, leafhoppers, white fly, and green stinkbug in cotton ecosystem. As a result of reduced insecticide use in Bt-cotton, beekeepers are keeping their bee hives (Apis mellifera) in Bt-cotton in northern India, and have not reported any negative effect of Bt-cotton on the bee colonies. In general, there are no adverse effects of Bt-cotton on the arthropod diversity, nontarget insect pests and natural enemies in the cotton ecosystem. However, regular monitoring of arthropod diversity and surveillance of important insect pests in the Bt-cotton ecosystem is necessary for sustainable deployment of transgenics for crop production.

Keywords:*Bt-transgenic, cotton, nontarget organisms, arthropods, ecosystem, monitoring*

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Effects of Cry1Ac Rice on *Propylea japonica* (Coleoptera: Coccinellidae), *Pirata subpiraticus* (Araneae: Lycosidae) and *Pachygnatha clercki* (Araneae: Tetragnathidae).

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The study was conducted to assess the potential adverse effects of a Bt-rice line (japonica rice cultivar Nakdong) expressing a synthetic cry1Ac1 gene, C7-1-9-1-B, which was highly active against all larval stages of Cnaphalocrocis medinalis (Guenee) (Lepidoptera: Pyralidae), on two spiders, Pirata subpiraticus (Bosenberg et Strand) and Pachygnatha clercki Sundevall, and a pollen-feeding predator, Propylea japonica (Thunberg). In the laboratory, P. subpiraticus and P. clercki were supplied with Bt and non-Bt (isoline) rice-fed brown plant hopper Nilaparvata lugens (Stal) and P. japonica was provided with one of three diets: Bt rice pollen with aphid Aphis gossypii Glover, non-Bt rice pollen with aphids, and aphids only. For P. subpiraticus and P. clercki, no negative effects were found on its survival, development, fecundity and adult physical characteristics. For P. japonica, the development time of larvae in the aphid only treatment was significantly shorter than in the two treatments where aphids were provided together with Bt or non-Bt rice pollen, but the survival, development and adult weight were not significantly different between Bt and non-Bt rice pollen with aphids. In a 2-yr field trial, populations of the three predator species were surveyed with a sweep net and suction device throughout the rice growing season in 2007 and 2008. The data show that Bt rice did not significantly affect the population density of P. subpiraticus, P. clercki and P. japonica. The results indicated that Bt rice expressing Cry1Ac had no significant adverse effects on the fitness and the population density of three important predators.

Keywords:*Bt rice, Cry1Ac, non-target effect, natural enemy, Pirata subpiraticus, Pachygnatha clercki, Propylea japonica*

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Assessing the impact of GE plants on natural enemies ? testing practices with *Coleomegilla maculate*

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To evaluate the potential effects of Cry2Ab- and Cry1Ac-expressing cotton on a ladybird predator Coleomegilla maculata (DeGeer), a tritrophic bioassay was conduced using Bt-susceptible and -resistant larvae of Trichoplusia ni as prey. The survival, development time, adult weight and fecundity were not different when C. maculata were fed with resistant T. ni larvae reared on either Bt or control cotton. To ensure that C. maculata were not sensitive to the tested Cry toxins independent from the plant background and to add certainty to the hazard assessment, a tier-1 bioassay was developed where the beetle larvae were fed artificial diet incorporated with Cry2Ab, Cry1Ac or both at > 10 times higher concentrations than in cotton tissue. Artificial diet containing E-64 was included as a positive control. No differences were detected in any life-table parameters between Cry protein-containing diet treatments and the control diet. In contrast, larvae of C. maculata fed the E-64 could not develop to the pupal stage and the 7-d larval weight was significantly negatively affected. In both bioassays, the stability and bioactivity of Cry proteins in the food sources were confirmed by ELISA and sensitive-insect bioassays. Our results show that C. maculata is not affected by Bt cotton and is not sensitive to Cry2Ab and Cry1Ac at concentrations exceeding the levels in Bt cotton, thus demonstrating that Bt cotton will pose a negligible risk to C. maculata. More importantly, this study demonstrates a comprehensive system for assessing the risk of genetically modified plants on non-target organisms.

Keywords:*environmental risk assessment, E-64, non-target effects, tiered testing, Cry2Ab, Cry1Ac cotton*

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S803TU10

Pesticides, GM Crops, Resistance and Toxicology

Assessing the impact of bruchid-resistant transgenic legumes on parasitoids

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Bruchid beetles (Coleoptera: Chrysomelidae) are a major threat to stored cowpeas. Genetically engineered plants expressing insecticidal proteins might be a powerful tool to contribute to the efficient control of this pest complex. The gene coding for the α-amylase inhibitor-1 (αAI-1) from the common bean has successfully been introduced into a range of legumes to protect their seeds against bruchid attack. Whereas αAI-1-transgenic legumes appear to be highly resistant against some bruchid pests, such as Callosobruchus spp., other species are not affected by this insecticidal protein. Consequently, αAI-1-transgenic legumes need to be combined with other methods to provide a better control of the bruchid pest complex. This includes the regulation conferred by natural enemies such as hymenopteran parasitoids. The combination of αAI-1-transgenic legumes with biological control requires that the transgenic trait does not adversely affect the parasitoids. We present a conceptual model and an analysis plan to assess the risk of αAI-1-transgenic legumes to bruchid parasitoids and provide results on the impact of the insecticidal protein on this important group of non-target arthropods.

Keywords:*biological control, bruchids, non-target effects, risk assessment, transgenic crops*

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Developing a famework for assessing the risks associated with RNAi crops on non-target organisms

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Recent discoveries of RNA interference (RNAi) and related silencing pathways have the potential to revolutionize modern agriculture. Besides technical challenges, the lack of a formalized/standardized ecological risk assessment (ERA) procedure is the biggest obstacle to integrate RNAi crops into the IPM rotation. An essential component of the ERA of RNAi plants involves in vivo RNAi toxicity testing under a defined worst-case scenario of exposure. The studies are designed to answer questions directly pertaining to the risk of in planta RNAi to non-target arthropods that are at greatest risk of exposure because of a shared environment and common molecular targets. In this research, we focus on the western corn rootworm (WCR), Diabrotica virgifera virgifera LeConte, one of the most devastating corn pest species in North America and which in planta expression of WCR dsRNAs has been demonstrated to protect corn plants from larval feeding. This report describes the very first step of a tiered approach, which is to measure specific risk endpoints under the worst-case scenario. This early-tier test will provide information on whether the potential hazards of RNA transgenes can be transferred to NTOs representing different habitats and diverse ecological functions. We believe this risk evaluation framework will provide an important model for future ERA of RNAi-mediated pest management technologies.

Keywords:*RNAi crop, ecological risk assessment, RNAi toxicity test, corn rootworm, sequence similarity*

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Pesticides, GM Crops, Resistance and Toxicology

A strategy on countermeasure against further development of pyrethroid resistance of vector mosquitoes: Minimizing the use of pyrethroids by synergistic actions of formamidines and by introducing new

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The current strategy for control of vector mosquitoes of human diseases relies heavily on the use of pyrethroids, inviting increasing resistance problems. Here we tested the possibility of using formamidines, amitraz and chlordimeform, as synergists to increase the effectiveness of pyrethroids and neonicotinoids for the purpose of controlling the 4th instar larvae of Aedes aegypti, a vector for dengue disease. Amitraz was found to show consistently more potent synergistic effects than chlordimeform. Against pyrethroids the synergistic larvicidal effects of amitraz was most apparent when larvae were co-treated with deltamethrin or fenvalerate type pyrethroids, but not with permethrin or fenitrothion. Against neonicotinoids, amitraz showed significant synergizing effects, particularly when they were co-treated with the newer type of neonicotinoids such as dinotefuran and thiamethoxam. To gain mechanistic insights we tested our hypothesis that formamidines cause hyper-excitation in mosquitoes through their agonistic action on the octopamine receptor (OR). We indeed found that amitraz consistently elevated the mRNA expressions of octopamine receptor (OR) and trehalase, which were most prominent in the presence of deltamethrin or dinotefuran. Furthermore, significant increases in free glucose levels were found when the larvae were treated with amitraz, which was greatly exacerbated when they were co-treated with deltamethrin, fenvalerate, dinotefuran or thiamethoxam, but not with permethrin, or fenitrothion. These results support our conclusion that the insecticides synergized by formamidines are the ones which cause neuro-excitation which is greatly exacerbated

Keywords:*Synergistic action of amitraz, pyrethroids, neonicotinoids, Aedes aegypti mosquitoes*

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Integrated measures for managing resistance for major pests in Malaysian crops

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The first official indication of insect resistance in Malaysia to insecticides was for the diamondback moth, Plutella xylostella, a major and cosmopolitant crucifer insect pest. Insecticide resistance for this pest was confirmed and quantified in 1978 and gradually it became an icon for resistant - incidentally to all major groups of insecticides, including the microbial Bacillus thuringiensis. Smith's disaster phase was averted by the timely intervention with an ecologically-based integrated pest management approach in the early 80s and 90s. However, recent escalations of this pest and that of another on rice, viz.,the brown planthopper, Nilaparvata lugens suggested maladies in the system. Recent farm surveys on pesticide use in both the crucifer and rice systems indicated the extensive array of pesticides used against the pests and the trying situations faced by farmers. Many cheap and illegal pestcides are also flooding the market. Malaysia's strategy towards sustainability is now under challenge from the the PULL effect of pesticides compounded by weak enforcement and the legislative PUSH towards non chemical approaches and proper stewardship programs for pesticides. The author shares experiences on the strategies used in mitigating or countering resistance and the options and challenges faced in the context of the current socio-economic-environmental scenario.

Keywords:*Pesticides, resistance, pests, Malaysia*

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Mechanisms of negatively correlated cross-resistance between malathion and fenvalerate in the brown plant hopper, *Nilaparvata lugens* Stal

Hayato Teshima[1], Tetsuo Saito[2], Tadashi Miyata[3]
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Hayato Teshima1,2, Tetsuo Saito1 and Tadashi Miyata1,3 1) Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya University, Chikusa, Nagoya, 464-8601 Japan 2) Present address: Health & Crop Sciences Research Laboratories, Sumitomo Chemical Co. Ltd., Takatsukasa, Takarazuka, Hyogo Prefecture, 665-8555 Japan 3) International Technical Cooperation Center, Rural Development Administration, Republic of Korea, Suwon, 441-707 Republic of Korea Successive selection with malathion in the brown plant hopper, Nilaparvata lugens Stal, resulted in the development of resistance to malathion but significantly susceptible to fenvalerate. On the other hand, successive selection with fenvalerate resulted in the development of resistance to fenvalerate but susceptible to malathion. Resistance to malathion might be caused by reduced cuticular penetration and enhanced degradation of malathion, and it was considered that higher susceptibility to malathion in fenvalerate selected strain might be caused by lower degradation activity of malathion by selection with fenvalerate. Cuticular penetration, in vivo metabolism and in vitro binding of fenvalerate were investigated. There were no differences in those mechanisms between malathion resistant and fenvalerate resistant strains. Mechanisms of decreasing of resistance to malathion with successive selection with fenvalerate remain subjects for future research.

Keywords:*negatively correlated cross-resistance, brown plant hopper, malathion, fenvalerate*

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An ace-1 expression system for monitoring insect acetylcholinesterase activity: With a special reference to carbofuran resistance in rice stem borer, *Chilo suppressalis* Walker

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Rice stem borer, Chilo suppressalis Walker, is one of the most important rice insect pests in Taiwan. Under heavy insecticide applications, more than 1000-fold resistance to carbofuran has been detected in certain field strains of this stem borer. Modified acetylcholinesterases (AChE) with A314S and H668P amino acid substitutions and increased Km and Vmax have been suggested to be associated with this resistance. The aim of this study is to verify the effect of these amino acid substitutions by expressing C. suppressalis AChE in Spodoptera frugiperda (Sf9) cells with the Bac to Bac Baculovirus Expression System. Full-length cDNA of ace1 obtained by RT-PCR was inserted into Bam HI/ Hind III sites of pFastBacTM 1 vector, which was then transformed into DH10BacTM Escherichia coli. The recombinant bacmids with ace-1 were isolated from E. coli and purified for the transfection of Sf9 cells. The recombinant viruses were harvested 96 hours post-transfection and collected as P1 viral stock. For further amplification, two more runs of transfection were carried out. Lysate of P3-infected Sf9 cells exhibited an AChE activity up to 32 ?OD min-1 mg protein-1 with 640 μM acetylthiocholine iodide. This result indicates that Bac to Bac Baculovirus Expression System is suitable and efficient for ace-1 expression. Our study provides a simple method to examine the linkage between the activities/amino acid substitutions of modified AChE and insecticide resistance development in insects.

Keywords:*ace-1 expression system, acetylcholinesterase, Chilo suppressalis, amino acid substitution*

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Resistance to mating disruption, a pheromone-based pest management tool, in the smaller tea tortrix, *Adoxophyes honmai* Yasuda

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Pest management using a copy of pheromone to interfere with sexual communication, termed mating disruption, is a possible substitution of insecticides. To date, many moths have been controlled with applications of formulated pheromones as mating disruptants. Repeated treatment with a disruptant, however, could theoretically impose strong selection on the targets, leading to evolutionary change of the pheromone-mediated communication and thereby potentially reducing the efficiency of this approach, as is the case with other insecticides. Indeed, the first example of resistance to mating disruption was documented in the smaller tea tortrix, Adoxophyes honmai, in Japan. To avoid other such cases, we challenged to elucidate why the disruptant was inefficacious. First, we imposed further selection by rearing the resistant colony with the disruptant in the laboratory. The active ingredient in the disruptant was (Z)-11-tetradecenyl acetate (Z11-14:Ac), which is one component of the sex pheromone of A. honmai. After more than 70 generations of selection, a strain with quite strong resistance was established, males of which could find their mates even in the presence of 1 mg/liter of Z11-14:Ac. We then investigated pheromone perception and production of this stain and uncovered that male response to the pheromone blend was markedly extended after selection; the resistant strain males could locate a synthetic pheromone source even when it lacked Z11-14:Ac that is normally indispensable for attracting A. honmai males. Males with the ability to lock on to an off-ratio pheromone blend may be more likely to overcome some of the disruptive effects of pheromone applications.

Keywords:*sex pheromone, mating disruption, resistance, artificial selection*

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S804W06

Pesticides, GM Crops, Resistance and Toxicology

The ultimate strategy on countermeasure for insect resistance to insecticide

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Since 1913 San Jose scale developed resistance to lime sulfur, many researches have been conducted but continue this problem. The origin of resistance is biological adaptation. Dvelpoment resistance has three phases, slow, hasty and stable. The ultimate strategy on countermeasure for insect resistance is insecticides rotation with no cross resistance insecticides with continual field monitoring. At present novel excellent insecticides developed. I hope those insecticides must carry out with judicious application.

Keywords:*adaptation, rotation, no cross resistance, judcious application*

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Global registration considerations for in planta RNA-mediated control of insect pests

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RNA based biotechnology-derived crops have been commercially available and safely consumed for over a decade. Global approvals for products such as virus resistant papaya, potato, bean, and squash, high oleic soybean, FLAVR SAVR tomatoes, and plum trees resistant to plum pox virus have all concluded that these products are safe for food and feed purposes using the current safety assessment paradigm for other biotechnology-derived crops. The RNAi pathway is a conserved, ubiquitous mechanism found in all multicellular organisms investigated thus far. Foods and feeds contain diverse populations of endogenous small RNAs (sRNAs) originating from plants and animals and are a normal part of human and animal diets. Many of these small RNAs are identical to vital mammalian genes and safely consumed. The next generation of RNA based products will utilize dsRNA to elicit a gene suppression response in pest species when produced in planta. The existing GM crop safety assessment paradigm is appropriate for evaluating potential unintended effects or hazards for RNA-mediated insect protected crops and additional studies are determined on a case by case basis to address product specific issues.

Keywords:*RNAi, global registrations*

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S805TH02

Pesticides, GM Crops, Resistance and Toxicology

RNAi products platform for invertebrates' health and targeted pest control

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Ribonucleic Acid interference (RNAi) applications for invertebrates are on the scientific agenda since RNAi was initially introduced. The work done on C. elegans not only was awarded with the Nobel Prize in 2006, but also triggered many initiatives that focused on how the RNAi mechanism can be used for pest management. RNAi based products are been developed by Beeologics to control honeybee viruses and parasites. The first and most advanced product to be introduced is Remebee® ? an anti-viral agent fed to the bees protecting them from acute infection caused by the Israeli Acute Paralysis Virus (IAPV). This virus has associated with honeybee mortality and identified as one of the potential participating factors of Colony Collapse Disorder (CCD). The technology platform built in the creation of Remebee covers many of the aspects of new product introduction and now supporting the R&D of several complementary RNAi products; for the honeybees as well as a variety of application for targeted pest control.

Keywords:*invertebrates' health*

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Developing species-specific RNAi technologies to control insect persts

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A serious problem for many insecticides is that they can adversely affect or even kill non-target species. In an effort to develop safer, less broad-spectrum pesticides, we have been harnessing the sequence-specificity of RNA interference (RNAi) to develop insecticidal double-stranded RNAs (dsRNAs) that selectively kill pests without impacting others. By targeting either highly divergent, rapidly evolving genes, or the 3' untranslated regions of more conserved genes, we have been able to develop dsRNAs that selectively killed only one species, while leaving closely-related species unaffected. We have been examining several methods of delivery of dsRNAs to insects, and have developed a variety of formulations to feed dsRNAs to a broad range of insects. Despite the apparent lack of dsRNA-specific receptors in dipertans, we have observed potent RNAi and effective pesticidal activity of ingested dsRNA in flies and mosquitoes; some of the endocytotic mechanisms used to facilitate dsRNA uptake in these insects have been identified and their relevance to RNAi in these and other insects will be discussed. We are also working on developing new formulations of dsRNA that may prove more environmentally stable, so that they could be more effective in field applications. While organisms may have thousands of genes that could potentially be targeted by RNAi, choosing the most effective dsRNA and the method of delivery of dsRNA could require some optimization for each pest species, and while this may prove daunting, the benefits of producing safer, more-specific pesticides are worth the effort.

Keywords:*RNAi pesticide*

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S805TH04

Pesticides, GM Crops, Resistance and Toxicology

Application of RNAi toward insecticide resistance

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RNA interference (RNAi) is a breakthrough technology for conducting functional genomics studies and also as a potential tool for pest management against insecticide resistance. On one hand RNAi acts as a functional genomics tool to study the molecular basis of physiological functions of target genes associated with insecticide resistance. A brain specific expressed Cytochrome P450, CYP6BQ9 was discovered from the deltamethrin resistant QTC279 strain of Tribolium castaneum. Functional studies using both double-strand RNA (dsRNA)-mediated knockdown in the expression of CYP6BQ9 and transgenic expression of CYP6BQ9 in Drosophila melanogaster showed that CYP6BQ9 responsible for the majority of deltamethrin resistance in the QTC279 strain. On the other hand ingestion bacteria synthesis dsRNA could be used as an application approach to manage insecticide resistant in agricultural important pests. In Leptinotarsa decemlineata, NADPH P450 reductase (CPR) which serves as the partner of all P450s was chose as the target gene for ingestion RNAi. Knockdown the target gene not only significantly decreased the imidacloprid resistant level in adults of imidacloprid resistant strain but also caused dramatically decreased number of eggs laid by adults and the stuck of embryonic development in eggs. These data suggest that ingestion of bacteria synthesis dsRNA of CPR could be used for crop protection against insecticide resistant pests.

Keywords:*Functional genomics, cytochrome P450, NADPH P450 reductase, bacteria synthesis dsRNA*

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Promoted transmission of silencing signals from plant to insect midgut by proteases

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Oral ingestion of plant tissues expressing double stranded RNA (dsRNA) resulted in target gene suppression in insect; this offers a potential approach against pest. Previously we reported cotton bollworm (Helicoverpa armigera) fed on transgenic Arabidopsis and tobacco plants expressing dsRNA targeting a bollworm P450 gene, CYP6AE14, exhibited suppressed expression of CYP6AE14 and reduced growth on gossypol-containing diet. We then transferred the dsRNA construct into cotton (Gossypium hirsutum) plants. Growth of the bollworm larvae reared on T2 cotton plants was drastically retarded, and the transgenic plants were less damaged by bollworms than the control. Accordingly, transcripts and protein levels of CYP6AE14 were reduced in the larvae fed on the transgenic plants. The key steps of this method are the production of ingested dsRNAs in plants and the spreading of these silencing molecules into the midgut of insect. Peritrophic matrix (PM), a chitin and glycoprotein layer lining in invertebrate midgut epithelium, presents a barrier preventing dsRNA molecules into midgut. We found that adding proteases into artificial diet elevated midgut permeability and enhanced RNA interference (RNAi) effect in bollworms triggered by plant-expressed dsRNAs. Similar results were observed when transgenic Arabidopsis overproducing plant proteases were used for insect feeding test. We have reported that CYP6AE14 is required for gossypol resistance of bollworm. We show that cotton plants co-expressing dsCYP6AE14 and protease were better protected from bollworm than single transgene lines due to enhanced RNAi effects in the bollworm. Our study demonstrated that RNAi technology for plant protection can be optimized by co-expressing specific proteases.

Keywords:*plant insect RNAi Peritrophic matrix proteases*

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Effective RNAi targets screening strategy and convenient dsRNA delivery way for Lepidoptera insect pest control

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To screen RNAi targets from Asian corn borer (Ostrinia furnalalis, ACB), second generation sequencing technology was adopted. Total 14690 stage specific genes were obtained which can be considered as potential targets, and 10 larval stage specific expression genes were selected for RNAi test. When 50 ng/μl dsRNAs of the 10 genes were directly sprayed on the newly hatched larvae which placed on the artificial diet, the mortalities were 73% to 100% at 5 d after treatment. The qRT-PCR analysis verified the correlation between larval mortality and down-regulation of the target gene expression. Topically applied fluorescent dsRNA confirmed that dsRNA did penetrate the body wall and circulate in the body cavity. To investigate whether the dsRNA has species specific or systemic effects in controlling insect pests by RNAi, a storage protein gene (OfSP) was identified from ACB. When the first instar larvae were sprayed with 50ng/μl dsRNA specific to OfSP, levels of the gene transcript decreased significantly. The result of OfSP gene silencing was the larval mortality of 52.54%, the alive larval will be abnormal pupation and failed to develop into adults. When the dsRNA of OfSP gene was sprayed on cotton borer (Helicoverpa armigera) larvae in the same dose, the correct mortality was only 3.6%. These results further confirmed that dsRNA indeed penetrated the insect body wall just by spray on the larvae and cause the systemic RNAi in the insects. It makes the RNAi technology can be easily used in the field of pest insect control.

Keywords:*RNAi, Target screening, dsRNA delivery, Species-specific.*

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S805TH07

Pesticides, GM Crops, Resistance and Toxicology

Controlling corn rootworm with dsRNA: Efficacy, specificity and IRM considerations

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The expression of dsRNA in crop plants, such as maize, to control damaging insect pests such as western corn rootworm (WCR, Diabrotica virgifera virgifera LeConte) represents a new class of insect control traits with a high degree of insect specificity. As with any new plant-expressed insect control trait, the potential for WCR to evolve resistance to dsRNA needs to be considered. This presentation will discuss information regarding pyramiding WCR-active dsRNA with Bt Cry proteins for increased efficacy and decreasing the potential for resistance development while maintaining a relative high degree of specificity in host spectrum

Keywords:*RNAi, Transgenic crops*

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S806F01

Pesticides, GM Crops, Resistance and Toxicology

Phytochemical variation: A limiting factor in pesticidal plant applications for small holder farmers in Africa.

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Recent surveys of small-holder farmers in Africa indicate that plants are still valued for insect pest control. However, we identified high levels of chemical variability in several pesticidal plants species which was unnoticed by farmers and led to widespread application of ineffective plant materials. We have shown that Tephrosia vogelii, a widely used pesticidal plant that is also grown in fallows to enrich soil, occurs as two distinct chemotypes. Chemotype 1 contained rotenoids, including deguelin and tephrosin which were responsible for the pest control efficacy whereas rotenoids were absent from chemotype 2, which was characterised by prenylated flavanones that are biologically inactive against the insects we tested. Sampling from 13 locations in Malawi where farmers cultivated T. vogelii for insecticidal use indicated that 25% of plants were chemotype 2 and so were unsuitable for this application yet were still being used for pest control. A second species under our study Lippia javanica has several pest control uses including against cattle ticks and the maize weevil Sitophilus zeamais. Activity depends upon perilaldehyde, however, this terpene was not present in all plants and where absent the major component was ipsdienone which was not toxic. Subsequent analysis of herbarium vouchers indicated that chemical variation was associated with different varieties that are possible sub-species and are difficult to distinguish. Meanwhile farmers continue using both sub-species without knowing which is effective. An understanding of the specific chemical variations and knowledge of active components is required to optimise propagation, harvest and use of pesticidal plants.

Keywords:*Tephrosia, bruchids, rotenoids, Lippia, Sitophilus, pesticidal plant*

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Variation between individuals in odour responses in *Callosobruchusmaculatus*: Effects of age, sex and morph

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Sex, morph and age may influence responses to behaviour-modifying odours in insects. The cowpea bruchidCallosobruchusmaculatusexists naturally in two adult forms: the active (flight) form for dispersal, and the inactive (flightless), more fecund but shorter-lived form. Given that these morphs show dissimilar biology it is possible that they differ in odour-mediated orientation. However, evaluations of pest control strategies that manipulate chemical ecology, such as the use of pesticidal plants or naturally deterrent crops, do not distinguish between them and rarely consider sex or age. We investigated whether the olfactory behaviour and orientation of C. maculatus towards infested and uninfestedcowpeas and a plant-derived repellent compound, methyl salicylate, differs between morphs or sexes. We found significant differences between the behaviour of male and female beetles and beetles of different ages, as well as interactive effects of sex, morph and age. This study demonstrates that behavioural experiments on insects should control for sex and age, while also considering differences between adult morphs where present in a species. This finding has implications for fundamental entomological research, particularly when exploring the relationships between physiology, behaviour and evolutionary biology, and the application of botanically-derived repellents as crop protection strategies.

Keywords:*Callosobruchusmaculatus*

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Optimisation of acaricidal activity of pesticidal plants *Lippia javanica* (Burm F.) Spreng and Maerua decumbens (Brongn) Dewolf against cattle ticks in Zimbabwe

Brighton Mvumi
University of Zimbabwe, Zimbabwe

The efficacy of 5% w/v Lippia javanica fresh leaves against cattle ticks after hot or cold water extraction; with or without liquid soap (surfactant) was evaluated on-station in comparison to commercial synthetic acaricide and water with surfactant. Mashona cattle, kept in separate paddocks per treatment, were used. Each animal was sprayed (5L) weekly for six weeks between January and February 2011. Prior to spraying and after every other day thereafter, full body tick counts on each animal were recorded. In laboratory bioassays, the effect of a surfactant plus hot or cold water extraction of the leaf powder of the pesticidal plant Maerua edulis applied at 5 to 25% w/v, was compared with solvent extraction. The on-station experiment showed a significant decrease ($p<0.05$) in tick population of different species across treatments. The reduction was higher ($p<0.05$) on cattle treated with L .javanica hot water extracts plus surfactant and the positive control (Tickbuster®) than cold aqueous extracts with surfactant only. In bioassays, there was a general reduction in total tick larval count post incubation in all treatments except the negative control (distilled water). Mortality was significantly higher in hot water plus surfactant treatments than it was in cold water plus surfactant treatments. There was no significant difference in tick larvae mortality between Tickbuster® and methanol-extracted M. decumbens. Use of hot water and liquid soap are low-cost and effective ways of optimizing the acaricidal efficacy of M. edulis and L. javanica and potentially other acaricidal plants under smallholder farming conditions.

Keywords:*Optimisation, pesticidal plant, Lippia javanica, Maerua edulis, cattle ticks, acaricidal efficacy*

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Larvicidal and acetylcholinesterase inhibitory activities of *Millettia pinnata* seed constituents toward insecticide-susceptible *Culex pipiens* pallens and *Aedes aegypti* and wild *Aedes albopictus*

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An assessment was made of the larvicidal and acetylcholinesterase (AChE) inhibitory activities of the four flavonoids karanjin (1), karanjachromene (2), pongamol (5), and pongarotene (6), and the two carboxylic acids (9Z)-octadecenoic acid (3) and hexadecanoic acid (4) identified in the seeds of Millettia pinnata toward third instar larvae from insecticide-susceptible Culex pipiens pallens and Aedes aegypti and wild Aedes albopictus. Results were compared with those of the conventional larvicide temephos. Karanjin (24 h LC50, 14.61 and 16.13 mg/L) was the most toxic compound, followed by (9Z)-octadecenoic acid, karanjachromene, pongamol, and pongarotene toward C. p. pallens larvae (18.07?25.52 mg/L) and A. aegypti larvae (18.45?25.76 mg/L). Hexadecanoic acid was least toxic (34.50 and 42.96 mg/L). Against wild A. albopictus larvae, (9Z)-octadecenoic acid (18.79 mg/L) was the most toxic compound, followed by karanjin (35.26 mg/mL). The LC50 of karanjachromene, pongamol, and pongarotene was between 52.97?64.97 mg/mL. Hexadecanoic acid (85.61 mg/mL) was least toxic. Overall, all the constituents were less toxic than temephos toward three mosquito species. No correlation was found between contact toxicity and AChE inhibition. Global efforts to reduce the level of highly toxic synthetic larvicides in the aquatic environment justify further study on M. pinnata seed constituents as potential larvicides or lead molecules for the control of mosquito populations.

Keywords:*Millettia pinnata, natural mosquito larvicide, flavonoid, carboxylic acid, acetylcholinesterase inhi*

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S806F05

Pesticides, GM Crops, Resistance and Toxicology

Insecticidal potential of plants in the Eurasian region - new opportunities for the development of botanical insecticides

Roman Pavela
Crop Research Institute, Czech Republic

Much attention is currently being paid to elimination of environmental and health risks associated with excessive and careless use of synthetic pesticides, not only in EU. Therefore major efforts are currently put into research and development of new environmentally safe preparations based on plant extracts ? the so-called botanical pesticides, which are considered safe for human health and for the environment. In several recent years the research has been also conducted in the Czech Republic with the objective to find new effective substances suitable for the development and production of botanical pesticides. The research focused on plants in the Eurasian region with the objective to identify plants whose extracts contain substances with insecticidal or fungicidal effects. The research involved not only screening of biological activity of plant extracts but, for the commercially usable extracts, also development of preparation formulas and optimization of the production. Some new preparations have been introduced on markets in selected EU countries in a relatively short time. This contribution briefly presents some results of the research dealing with the first stage of the research ? screening of extract effects on insects. It summarizes effectiveness of extracts, obtained from 250 plant species from the Eurasian region, in terms of mortality, antifeedancy and growth inhibition of insects. It also outlines some important problems that accompany the efforts to put results of the research into practice. (Project No. LH11133)

Keywords:*Botanical insecticides, Plant extracts*

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S806F06

Pesticides, GM Crops, Resistance and Toxicology

Recent trends in the use of botanical insecticides in North America

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The potential of plant extracts and plant natural products for insect management continues to be a subject of considerable research and scientific interest, but the interest from the agrochemical community and consequently the commercial adoption of botanicals lags far behind. Given the logistical and regulatory difficulties faced by companies that have attempted to bring new botanicals to market, it can be argued that more research should be directed toward optimal utilization of existing and recently introduced botanicals rather than the continued search for new plant extracts or plant natural products possessing bioactivity against pest insects demonstrated in laboratory bioassays. Recent use data from California suggest that the growth in use of botanical insecticides has been very modest, in spite of the substantial growth of large-scale organic food production in the state. Botanicals must compete with many more efficacious insecticides, including microbials and reduced-risk synthetic chemicals. In G20 countries, botanical insecticides will be more valued as consumer products than for professional or agricultural applications.

Keywords:*botancical insecticides*

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S901M01

Conservation, Biodiversity and Climate Change S901

Effect of climate change on the geographical distribution and abundance of insect pests

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Climate, especially temperature, has a strong and direct influence on insect development, reproduction and survival and is considered the dominant abiotic factor directly affecting exothermic organisms. It is expected that milder winters will increase the survival rate of pests and will allow a faster population recovery; an extended growing season could benefit multivoltine species to produce a larger number of generations and also increase the risk of invasive pest species; climate change might as well dissociate predator-prey relationships. Understanding and quantifying future shifts in pest range and damage is crucial for any strategy to better manage plant health. We applied temperature-driven pest phenology modeling and risk mapping in a Geographic Information Systems (GIS) environment to assess and understand pest shifts across regions. For simulations, mapping and the quantification of changes, we used the Insect Life Cycle Modeling (ILCYM) software recently developed by the International Potato Center, Lima, Peru. Downscaled future climate scenarios from SRES-A1B projections of the year 2050 were used. Results for several potato pests and related parasitoids will be discussed. As an illustration, the damage potential of the potato tuber moth Phthorimaea operculella, will likely increase in all regions where the pest already prevails today, with an excessive increase in warmer cropping regions of the tropics and subtropics. A possible range expansion into tropical temperate mountainous regions with a moderate increase of its damage potential is also predicted. This information is important to prepare farmers for such change and to develop adaptation strategies to climate change.

Keywords:*ILCYM*

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S901M02

Conservation, Biodiversity and Climate Change

Effects of climate change on the population dynamics of arthropods

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Climate change is one of the driving factors responsible for ecosystem regime shifts, and affects the pattern of population dynamics of arthropods through various ways. For example, global warming can cause a higher over-winter survival, earlier appearance in spring, an increase in the number of generations in a year, lengthening of the reproductive season and so on. Global warming mediated by increases in [CO2] also indirectly affects the development of herbivores through a change in plant quality, such as C/N ratio. The time span required for a regime shift ranges from one year to a decade depending on the life history traits and the distribution range, and the kind and intensity of the driving factors. As long as the regime remains unchanged and the intensity of the driving factor (s) does not overwhelm the regulatory mechanisms in the population, the affected population can recover sooner or later. Even a single-year change in climate, if it allows predators to outbreak, may be strong enough to cause an irreversible regime shift. This suggests that unexpected catastrophes may occur when the impact of climate change disrupts the mechanisms regulating the population. Most insects are susceptible to heat stress between 28 and 32oC, which is lower than what was commonly believed. Climatic change driven by human activities is predicted to lead to an increase of temperature between 1.4°C to 5.8°C by 2100. Thus, global warming could cause a more profound impact on the population dynamics and biodiversity of arthropods than has been previously documented.

Keywords:*driving factor, regime shift, regulatory mechanism, heat stress*

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S901M03

Conservation, Biodiversity and Climate Change S901

Impact of climate change on natural enemies of insect pests

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Numerous studies have addressed the effect of global change on physiology, life history traits and population dynamic of species, but few have included species responses in a network context, such as that relating preys and predators. We investigate whether climate changes impact the capacity of natural enemies, mainly parasitoids, to act as regulators of insect pests. Based on a review of the literature and our recent results, we highlight that parasitoids are expected to respond to modifications of the mean temperature and also to the increase of the thermal range, resulting from climate changes. First of all, the performance of adults and the relationship between their size and fitness are deeply affected by temperatures experienced during developmental stages. Secondly, adaptation to a larger thermal variability depends on the phenotypic plasticity and may lead to the selection for generalist strategies. In this case, costs of phenotypic plasticity result in a trade-off between maximum and breath of performance traits, like fecundity, decreasing the efficiency of parasitoids to face to high host densities. Finally, energy allocation between current and future reproduction relies on environmental variability and stochasticity, such that climate changes are expected to decrease the synovigenic index of parasitoids. Dynamic of insect pests and the emergence of new ones have to be analyzed and thermal challenges incurred by parasitoids should be taken into account in biological control programs.

Keywords:*parasitoid, phenotypic plasticity, life history traits, temperature*

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S901M04

Conservation, Biodiversity and Climate Change S901

Climate change effects on Insect - host plant - herbivore interactions

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Chemical composition of plants will change in direct response to global warming and climate change, affecting growth and development of insect pests. Moisture stress will lead to either an increase or decrease in insect damage. Water stressed pants, in general, are more susceptible to insect pests. Atmospheric humidity also interferes with insect plant interactions as it influences detection of host plant odors, and thus, may influence host finding and antixenosis mechanism of resistance to insects. Increased levels of CO2 will enhance plant growth, but may also increase the damage caused by some phytophagous insects. Increased CO2 may result in a slight decrease in nitrogen-based defenses (e.g., alkaloids), but increase the carbon-based defenses (e.g., tannins). Environmental factors such as soil moisture, and atmospheric temperature and humidity have a strong influence on expression of Bacillus thuringiensis (Bt) toxin proteins in transgenic plants. Genetically modified cottons with Bt toxin genes have lower nitrogen, and higher carbon-nitrogen ratio, carbon defensive compounds, condensed tannins, and gossypol under elevated CO2, which results in a significant decrease in Bt toxin expression. Decrease in relative humidity and increase in minimum and maximum temperatures increases the efficacy of transgenic cotton against the bollworms, but there is a significant decrease in biological activity once the minimum and maximum temperature are > 22 and > 38 oC, respectively. It is therefore important to understand the effects of climate change on the efficacy of host plant resistance to herbivores for pest management and sustainable crop production.

Keywords:*Climate change, plant herbivore interaction, biochemical changes, plant resistance, pest management*

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S901M05

Conservation, Biodiversity and Climate Change S901

Towards biodiversity-based ecological IPM: Case in US apple production

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The use of selective insecticides, pheromone mating disruption, and disease-resistant cultivars have the potential to move apple IPM towards sustainability. Conserving biological control agents and native pollinators can reduce the need for pesticides and a reliance on the honey bee for pollination, but rapidly changing arthropod communities in apple orchards and their dependence on features of the surrounding landscape need to be better understood. Regulatory mandates both in the U.S. and in the European Union are greatly restricting or eliminating the use of broad-spectrum insecticides which drastically reduced arthropod biodiversity in fruit orchards for over 50 years. We have documented increases in predator, parastioids and bee biodiversity that set a baseline for the development of a new level of IPM that is not pesticide driven. Ecologically-based IPM is based on understanding the biodiversity that makes up the ecological communities in what should be one of the most stable agroecosystems. We will discuss how climate change and invasive species could upset this stability.

Keywords:*Ecological IPM, Biodiversity, Apple Orchards, Pollination, Pesticides*

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Effect of climate change on bioefficacy of IPM technologies

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Host plant resistance, biopesticides, natural enemies and synthetic chemicals are some of the potential options for pest management, and the relative efficacy of many of these pest control measures is likely to change as a result of climate change. Global warming may result in breakdown of resistance to certain insect pests. Climatic change may also disrupt the balance between insect pests and their natural enemies. The natural enemy ? insect host relationship is influenced by both temperature and relative humidity, which are critical for survival and development of the immature stages of the parasitoids. Postembryonic development of parasitoids is prolonged at cooler temperatures, and the mortality increases at temperature extremes. Conversely, the arthropod predators are directly exposed to the prevailing environmental conditions and experience direct effects of the changing climatic conditions on development and predation potential. Temperature, humidity, elevated atmospheric CO2, and UV radiation also alter the rate of entomopathogenic fungi and nematodes entry into the host insect. Insect viruses are known to be inactivated by high temperatures, sunlight and ultraviolet rays. Sunlight, CO2, and pH exhibit varied influences on the toxicity of synthetic pesticides. Rainfall reduces the insecticide toxicity, however, it varies with the intensity and amount of rain, insecticide formulation and the adjuvants used. Therefore, there is a need to have a concerted look at the likely effects of climate change on the efficacy of different crop protection technologies, and devise appropriate strategies to mitigate the adverse effects of climate change on the bioefficacy of IPM technologies.

Keywords:*Climate change, IPM, insect pests, natural enemies, biopesticides, synthetic chemicals*

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S901M07

Conservation, Biodiversity and Climate Change S901

Impact of climate change on activity and abundance on natural enemies of insect pests

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Under climate change, the effectiveness of natural enemies in controlling herbivores will be influenced by changes in distribution of both pests and natural enemies. Natural enemies with effectiveness in controlling pests may decrease if pest distributions shift into regions outside the distribution of their natural enemies, unless a new community of enemies provides some level of control. Pest species with both native and invasive distributions may provide some insight into the likelihood of these scenarios happening. Mealybugs currently considered pests in Australian vineyards include two of Australian origin, long tailed mealybug Pseudococcus longispinus (Targioni Tozzetti) and citrophilus Pseudococcus calceolariae (Maskell), and an invasive, obscure mealybug P. viburni (Signoret); all three also occur in .many countries. At the same time there is concern for two species of invasive mealybugs of importance; vine Planococcus ficus (Signoret) and grape Pseudococcus maritimus (Ehrhorn) mealybugs, both also widely distributed, native to some affected areas and invasive to others. Comparisons of the parasitoid complexes associated with these mealybugs in their ranges of origin and in their invasive ranges provide an opportunity to predict possible outcomes. This talk discusses these possible outcomes and the types of data required to make improved predictions.

Keywords:*parasitoid, pest control, biodiversity, invasive, endemic*

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S901M08

Conservation, Biodiversity and Climate Change

Aspects of arthropod ecology : A contribution to sustainable agribusiness in South Africa

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The sustainability of an agribusiness depends on the conservation of its biodiversity. The development of a robust methodology that can be used in an Environmental Management System (EMS) is thus necessary by (i) implementing measures of arthropod and vegetation biodiversity and (ii) incorporating a monetary value to biodiversity, as mechanisms to indicate the degree of disturbance within the agro-ecosystem. Case studies were conducted to pinpoint potential indicators of an ecological nature, with specific reference to the biodiversity of arthropods and vegetation, as part of the methodology. Arthropods were used as an indicator community since they are prevalent, have high species diversity, are easy to sample, are important in ecosystem function, provide early detection of ecological changes and respond to environmental changes faster than vertebrates. The influence of agricultural management practices on the biodiversity is also taken into consideration. Quantitative and qualitative analysis revealed distinct patterns within the biodiversity and is observed at the different locations, relative to the frequency and diversity of vegetation sampled. Thus aspects of ecology (edge-effect, guild structure, biodiversity, etc.) should indicate a relationship between communities, indicating the level of ecosystem integrity. This level of ecological integrity could be taken into consideration when adopting a quality EMS. Possibilities of incorporating a monetary value to biodiversity, that in turn have a bearing on ecosystem services and agribusiness is also addressed. An EMS bio-indicator protocol is important as an aid to reduce the impact of agricultural management practices on the agro-ecosystem and to increase its functional efficiency.

Keywords:*Biodiversity, Environmental Management System (EMS), agro-ecosystem, indicators, ecology*

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S901M09

Conservation, Biodiversity and Climate Change

Insect biodiversity: Created dung beetle communities in pastures

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Species-rich dung beetle communities (Scarabaeinae) have evolved in complex habitats such as tropical forests, and tropical savannahs. These communities are sensitive to human disturbance of the habitats and heavily-modified habitats like improved pastures are typically very species-poor. In the natural forests, woodlands and heath of Australia there is a rich dung beetle fauna, but in nearby improved pastures grazed by cattle the dung beetle community is impoverished. Exotic scarabaeine dung beetles were introduced to pastures in Australia from 1968-1984 to create a community that provides ecosystem services with increased burial of cattle dung throughout the year. The seasonal abundance of the newly created community is described for the agricultural regions of SW Australia which has a cool wet winter growing season and a warm dry summer. The endemic dung beetle species are winter-active. The summer-active introduced species had spread and became abundant by 1993 after 15 years. The summer-active species have two generations a year whereas winter-active species have one generation a year. Winter-active species have spread and become abundant in 2012 after 30 years. This created dung beetle community of 10 species now has a seasonal pattern of beetle biomass similar to that of the natural communities that have evolved in the complex more fragmented woodland and open native pasture habitats found in regions of southern France and Spain with a similar climate.

Keywords:*Scarabeine dung beetle community*

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S902F01

Conservation, Biodiversity and Climate Change S902

Using ecological networks to conserve arthropod biodiversity in transformed landscapes

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Understanding edge effects between transformed landscapes and conservation areas is critical to conservation planning. Conservation areas in landscape mosaics are often composed of linear corridors, resulting in more edges than would occur naturally. Arthropods are the focal taxa for this study as they are abundant, diverse, and environmentally sensitive at point localities and so represent a wide range of taxa and their responses to anthropogenic change. Plantation forestry has transformed much of South Africa's grassland, with ecological networks (ENs) currently being used to mitigate this biodiversity loss and to maintain ecosystem processes. I compare arthropod biodiversity along transects that ran from within plantation blocks or indigenous forest patches (as a natural reference) into indigenous grassland corridors or into protected areas. Two types of exotic commercial trees and various tree age classes were studied. I found a 32 m edge zone from plantation blocks into grassland corridors. Few significant edge effects from plantation blocks occurred at greater distances than this, suggesting that grassland corridors with a width <64 m are mainly edge. This situation is complex, with different taxonomic groups responding differentially to these edges, although by 32 m from the plantation blocks, arthropod richness and species composition was similar to those of neighbouring protected areas. Indigenous forest supported many additional species, not just within the forest, but also with associated grassland corridors. This means that natural edges are important for maintaining biodiversity, while linkages within transformed habitats need to be wide enough to overcome edge effects.

Keywords:*Ecological Networks, Landscape Ecology, Conservation, Biodiversity, Edge Effects*

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S902F02

Conservation, Biodiversity and Climate Change S902

Arthropod biodiversity in Ethiopia - Religion and science partner for conservation

Margaret Dalzell Lowman
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Only four percent of Ethiopia's original forests remain, and most of these are small forest fragments surrounding the Coptic churches, many over fifteen hundred years old. As stewards of these last forest tracks, the Ethiopian priests are just beginning to understand the critical ecosystem services provided by these 'church forests' including fresh water springs, pollinators, medicines, honey, biodiversity, and the country's cultural/spiritual heritage. Over the past four years, teams of entomologists have collaborated on arthropod biodiversity surveys of these church forests, prioritizing insect pollinators and canopy biodiversity. In addition to many discoveries of new species and their ecological roles, we have conducted important education outreach to the local children about insects, and worked closely with the church leaders to foster a conservation ethic. The church represents a relatively diverse stakeholder in global forest conservation, but with the strong support of the priests and their parishoners, stone walls have been constructed and restoration of native species is well underway. Continued efforts to classify the arthropod diversity, and education outreach to Ethiopian children, have fostered a new conservation paradigm. The complexities of studying species diversity, in addition to the urgent priorities for habitat conservation and ecosystem services, have led to innovative new models of conservation action plans where economics, ecology, taxonomy, and community sustainability must all become part of the solutions.

Keywords:*insect biodiversity conservation*

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S902F03

Conservation, Biodiversity and Climate Change S902

Linking forest biodiversity to sustainable livelihood through commercial insects in Africa

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African forests, which are host to some of the world's richest biodiversity, are rapidly diminishing together with their local flora and fauna. The loss of flora and fauna includes economically and socially important insects, such as honeybees and silkmoths. Grouped under commercial insects, they are a source of insect-based enterprises that provide income to forest-edge communities, thus helping to manage this ecosystem. The realized income is reported to be having a direct impact on households through increased ability to fund children's education and health delivery. The project activities link beekeeping and silk production to Participatory Forest Management (PFM) initiatives in Kenya and several other forests in East and North Africa. The commercial insects enterprises are particularly suitable for use in buffer zones around protected forests. The project also highlights the importance of pollination services in Africa and alternate nutritional sources from agricultural waste. The project has initiated efforts to maintain the health of bees, in view of the recent unexplained disappearance of bee colonies around the world. This seminar will also highlight icipe's and AU-IBAR's recently approved project by the European Commission entitled: African reference laboratory (with satellite stations) for the management of pollinator bee diseases and pests for food security. A central reference laboratory (CRL) will be established at icipe Kenya, as well as four satellite stations (one each in Ethiopia, Cameroon, Burkina Faso and Liberia), to generate new knowledge on bee diseases and pests and their control, for Farmers' Federations (FF) and beekeepers in general.

Keywords:*Commercial Insects, Bee Health, Ecosystem services*

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S902F04

Conservation, Biodiversity and Climate Change S902

Butterfly biodiversity in the Australian Monsoon Tropics: A preliminary study

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The Australian Monsoon Tropics biome comprises a vast wilderness region across northern Australia with relatively intact savannah landscapes, and includes the Kimberley and Top End as major subregions in the north-west. It is a frontier for biological research and species discovery. The diurnal Lepidoptera (butterflies and day-flying moths) have been selected as one of several surrogate groups to estimate patterns of invertebrate biodiversity within the region. This study seeks to understand the species richness (alpha-diversity), composition and spatial turnover (beta-diversity), spatial distribution and extent of endemism, and threatening processes within the Kimberley?Top End?western Gulf Country. The aim is to use this information to identify sites of conservation significance, and assess the conservation status of each species, with the long-term goal of determining the extent to which invertebrate biodiversity is represented and protected under the current National Reserve System, and to provide recommendations for future reserve design/conservation management. Recent surveys in the region have revealed a number of remarkable taxonomic discoveries that suggest that the level of endemism is higher than previously realised. Attention is briefly drawn to several of these discoveries, one notable example being the presence of a species of Delias which is restricted to tropical mangrove habitats in coastal estuarine areas where it breeds on Euphorbiaceae. The unique habitat association, novel food plant specialisation and narrow geographical range of D. aestiva underscores the biogeographical peculiarities of north-western Australia, especially patterns of historical differentiation between the Top End and Cape York Peninsula within the Australian Monsoon Tropics.

Keywords:*Australia, Biodiversity conservation, Butterfly, Delias, National Reserve System, Pieridae*

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S902F05

Conservation, Biodiversity and Climate Change S902

Identifying biome switches by describing the relative role of dispersal vs. environment in structuring ant assemblages

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There has been widespread documentation of woody thickening of mesic savannas across southern Africa. Beta diversity patterns and structure of assemblages in stable environments would be expected to be under environmental niche control, whereas communities in changing assemblages would be dominated by neutral processes. We test this ascertain by determining the relative roles of dispersal and environment in generating beta diversity patterns of ant assemblages on two aspects (xeric and mesic) of the Soutpansberg mountains in South Africa. Ants were sampled with pitfalls traps at 11 altitudinal zones across the mountain. Sixty three percent of the variation in ant assemblage structure on the northern (arid) aspect was the result of environmental sorting processes whereas neutral processes accounted for considerably more of the variation on the southern (mesic) aspect (24 %) than on the northern slope (11%). Ant assemblage composition in sites with historical evidence of thickening is similar to that of the tall forests on the mountain. Ant diversity was also the lowest in sites characterised by thickening. These negative consequences are also predicted for other invertebrate taxa. Global climate change predictions suggest that rainfall in the region will increase by ca. 30%. This could result in increased and widespread thickening with important consequences for the maintenance of savanna species.

Keywords:*Savanna, thickets, climate change*

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S902F06

Conservation, Biodiversity and Climate Change

Restoring ecological integrity in depauperate agro-ecosystem soils : Disentangling the underlying issues

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Understanding ecosystem function is pivotal in maximizing outputs in human driven agricultural production systems. Whilst the activities of the above ground components of such systems are well-documented, the below ground biotic community assemblages and their underlying interactions have only relatively recently received more in depth attention. Important in agro-ecosystem function is that, whilst well-established ecological principles apply, several issues deviate from the norm. Central in this context are anthropogenic disturbance and subsequent succession tempos which are often under-emphasized and which need special attention in any agro-ecosystem analysis scenario. Insects, as part of the larger arthropod biodiversity pool in the soil, fulfil a meaningful role in this medium because of their crucial linkages on different levels of the larger trophic structure. In order to provide a reference base for discussion, this paper will refer to the composition and function of various hexapod groups in this regard. Underlying aspects that will receive attention include temporal variability, and landscape scale and heterogeneity, as drivers of change; mismatch between agricultural management and ecological processes; contribution of plant-soil feedback loops in biodiversity enhancement; soil enrichment and the home-field advantage principle; functional shifts and stratification; meta-communities and the rescue-effect principle; site history; and the ecological integrity, agricultural landscape planning and sociology connection. Finally, to address depauperate soil recovery and mitigate soil degradation in agro-ecosystems, recognition of multifunctional agricultural landscapes and integrated adaptation management practices, based on the adaptation models of Holling & Gunderson (2002) and Meinke et al. (2009), are discussed.

Keywords:*Soil ecology*

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S902F07

Conservation, Biodiversity and Climate Change S902

Entomology research after the convention on biological diversity

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A major snag of the Convention on Biological Diversity (CBD) is a shift in focus from the ecological and scientific value of biodiversity to its commercial value. Articles 3 and 15 of the CBD recognize the sovereign rights of nation states over their biological resources and their authority to determine access to genetic resources through national legislation. Most countries have developed legislations and implementing mechanisms to regulate access to genetic resources within their national boundaries . Unfortunately, the national implementing mechanisms of the CBD decisions, in the hands of the bureaucracy in several countries, have made international collaborations nearly impossible in the fields of systematic, plant protection, management of invasive species and conservation. Most taxa do not have any direct commercial importance and research in basic sciences like taxonomy does not yield any monetary returns but significantly advances the horizons of knowledge. It is rather perplexing to note that the Rules and guidelines framed by the nation states to implement Convention on Biological Diversity , are being irrationally imposed on fundamental research and curtails the scientific freedom. Hence the science community should try to get academic research exempted from the national access and benefit sharing (ABS) regimes under the CBD

Keywords:*sovereign rights, genetic resources, scientific freedom, access and benefit sharing*

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S902F08

Conservation, Biodiversity and Climate Change

Arthropod function and redundancy: A baseline for monitoring environmental change in naturally fragmented habitats across disturbance regimes

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Biodiversity is a crucial but complex concept in conservation ecology. The comprehensive evaluation thereof is challenging and therefore numerous approaches have relied on indicators to interpret selected elements of biodiversity. Arthropods have been found to be the most successful indicators because of their significant role in the overall function of the ecosystem and their sensitivity to environmental change. The indicator approach has however shifted from focussing on single species, abundance and rarity, to incorporating more than one species, different levels of ecosystem functionality and structure, as well as landscape structure. Landscape heterogeneity in particular has been found to play a crucial role in arthropod ecology and dispersal. Mosaic habitats have shown to host significantly high biotic diversities, however most research in this field has focussed on large-scale human induced fragmentation and limited information is available on the functioning of smaller-scale naturally fragmented habitats. This study employed arthropod feeding functional groupings as a tool to indicate ecosystem resilience between naturally fragmented limestone-associated vegetation units in the Eastern Cape province of South Africa. We investigated arthropod relationships across three disturbance regimes and their spatial entities to understand the role of habitat structure in arthropod community structuring and functioning. Locally, this study provides a baseline understanding of the important functions and interactions at play in the Eastern Cape limestone-associated vegetation units for monitoring environment change. Globally, it creates a platform for comparison with limestone associated landscapes, and serves as a model for how to approach biodiversity conservation in small-scale naturally fragmented mosaic units.

Keywords:*Biodiversity conservation, Mosaic landscape, Calcrete*

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S902F09

Conservation, Biodiversity and Climate Change

Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring

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Traditional biodiversity assessment is costly in time, money and taxonomic expertise. Moreover, data are frequently collected in ways (e.g. visual bird lists) that are unsuitable for auditing by neutral parties, which is necessary for dispute resolution.? We present protocols for the extraction of ecological, taxonomic and phylogenetic information from bulk samples of arthropods. The protocols combine mass trapping of arthropods, mass-PCR amplification of the COI barcode gene, pyrosequencing and bioinformatic analysis. We perform an experimental test and show that we can recover a substantial proportion of the original taxonomic information. We further demonstrate that metabarcoding allows for the precise estimation of pairwise community dissimilarity (beta diversity) and within-community phylogenetic diversity (alpha diversity). Finally, we present applications of metabarcoding to tropical biodiversity censuses. Alpha and beta diversity metrics are the raw materials of ecology and the environmental sciences, facilitating assessment of the state of the environment with a broad and efficient measure of biodiversity.

Keywords:*DNA barcoding, metagenetics, biomonitoring, phylogenetic diversity*

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S902F10

Conservation, Biodiversity and Climate Change S902

Conserving pollinators: Can standard restoration mixes restore pollinator diversity?

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Conserving native pollinators by restoring native plants is considered critical to preserving their valuable ecosystem service. However, this assumes standard restoration plants are preferred foods for pollinators. This is despite restoration plants being chosen for a number of reasons aside from pollinator diet including plant community structure, seed availability, ease of planting and viability within a restoration. We determined if standard prairie mixes could aid in conserving a significant number of pollinator species. Using extensive records on plant-pollinator interactions in remnant Iowa prairies, we simulated the bee communities that could assemble using standard prairie mixes. We also simulated the bee communities that would assemble if alternate methods were used to choose plants such as plant species abundance in remnant prairies and plant species that had higher bee species associations. Although prairie mixes offer an easy method to begin restoring native plant diversity, species accumulation curves showed that these plant species accumulated fewer bee species at a much slower rate than choosing plants based on bee associations or abundance in remnant prairies. Interestingly, five additions to standard prairie mixes could restore an additional 50% of bee species. Some premier prairie mixes included some of these bee attractive species. This suggests that if money is spent to choose more expensive and more diverse prairie mixes there is a greater chance of success for attracting pollinators. In conclusion, although restoration could improve native bee conservation more targeted efforts need to be made to succeed in conserving pollinators.

Keywords:*pollinator, conservation*

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S902F11

Conservation, Biodiversity and Climate Change S902

Elevational pattern in diversity of butterflies in the Western Himalayas, India

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We studied elevational pattern in butterflies (Lepidoptera: Rhopalocera) species richness and diversity in Tons valley, Western Himalayas. We test a number of hypothesis that were proposed as 'general explanations' for these patterns namely (1) mid-domain effect, (2) overlap between butterfly faunal compositions, (3) water and energy availability and (4) area of elevational bands. Line transects and direct search approaches were used to quantify local species richness and diversity (Fisher's α) at 130 sites during 2009 - 2011. As complete lists as possible have been complied for each 100 m elevational band, starting from an elevation of 900 - 3500 m. For each of the 26 elevational bands, climate and NDVI data have been acquired and plot level microclimatic, vegetation and disturbance parameters were also quantified. A total of 174 butterfly species were recorded and two distinct, lowland and upland assemblages were found. Result shows an overall decreasing pattern in species richness, abundance and diversity with increasing elevation, while evenness was high at higher elevations. Peak species richness and diversity was observed between 1200 - 1500 m. The observed patterns differed significantly from mid-domain null predictions. Area supported an increase in species richness upto mid elevations, while species richness seemed to be controlled largely by climatic variables in high elevation zones. Strongest vegetative predictors of butterfly richness were plant species richness, herb and shrub density. We also evaluated a number of parameters of conservation value and identified sites of most conservation potential in the Himalayan landscape.

Keywords:*Mid-domain effect, Geometric constraints, Garhwal Himalayas, Elevational gradient, Species richness*

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Crop pollination services foster rich native bee biodiversity and abundance in western USA

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Conversion of native habitats to crop production is believed to have led to population declines of native bees, especially bumble bees, due to losses in foraging resources which they require over several months for growth and development. However, native bee studies in cropping systems have largely focused on a single crop. Here, our objectives were to determine native bee diversity and abundance in two crops that bloom in sequence. The study was conducted in the agriculture dominated Willamette Valley in western Oregon on the west coast of the United States in commercial fields of spring blooming blueberries and red clover seed crops which bloom in summer. Over 30 native bee species belonging to 10 genera in 5 families were recorded in bee traps. These included six bumble bee species which were also abundant in timed visual counts made in both crops. Bumble bees captured/day increased from 6/trap in blueberries to >30/trap in red clover. Based on visual count data, an average of 1 bumble bee/min foraged on blueberries and 15-30 bees/min on red clover during peak bloom. Thus, in contrast with reports from other regions, a rich fauna of native bees, especially bumble bees, is flourishing in western Oregon. Native bee populations are likely sustained here through the practice of farming bee-pollinated crops that bloom in sequence, and in synchrony with foraging by queens and workers. Based on our studies, we recommend production of multiple crops that bloom in sequence for sustaining native bees in agricultural landscapes.

Keywords:*bumble bees, agricultural landscapes*

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S903F01

Conservation, Biodiversity and Climate Change S903

Insect biodiversity: More than just bugs under climate

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Insects are not just 'pest' or 'beneficiary' but six-legged animals, the largest block of global biodiversity, 58-67% of global biodiversity with more than million species so far described that evolved for over 400 million years. Insects are highly resilient and closely associated with humanity as new habitats and hosts are opened up for insects almost everywhere and they are highly resilient for survival. As biodiversity refers to the totality of living organisms at a spatially defined unit, insect biodiversity signifies that insects are not only most species rich and most abundant taxon but also important ecological partners vital to sustaining ecosystem functions. Biodiversity loss has continued without even knowing what they were which would be compounded by the effects of global warming and climate changes. Considering that biodiversity is fundamental to ecosystem function and also primary natural resource for our life-support system the inventory of site-specific biodiversity could provide basic biodiversity database for the target community would provide a baseline information on the species composition of its biodiversity which would help advance sustainable development at the grassroots. This paper will also show how this process is accomplished and how biodiversity and ecological information could be directly applicable to pest management, ecosystem management, and land-use planning along with conservation and application of local biodiversity to local economic and natural resource development. To do so also shows the need for a taxonomic/biodiversity infrastructure for species identification and biodiversity assessment.

Keywords:*Insect biodiversity, biodiversity concept, taxonomy, inventory, assessment, taxonomic services*

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S903F02

Conservation, Biodiversity and Climate Change

Will climate change and biological invasions act synergistically? Response from the ants

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Ants are among the most devastating invasive species and extremely difficult to control. They can have enormous impacts on native biodiversity and ecosystem functions. In addition, it is generally feared that climate change will favour invasive organisms, especially poikilotherms such as ants, by removing thermal barriers that currently prevent them from invading cooler regions In this study, we compare the potential habitat of 15 of the worst invasive ant species today and following climate change. The species' potential habitat has been estimated using ecological niche models (ENMs) based on 5 different modelling techniques, 3 Global Circulation Models and 2 CO2 emission scenarios, which have been combined in an ensemble forecast yielding a consensual model. Our results are unexpected and question the dogma of the synergism between climate change and invasive species. In brief, there was no unique trend for all of the 15 ant species, with several species increasing in invasive range, several others maintaining a similar range while several species even decreased in invasive range. In addition to this heterogeneity at a global scale, there was a considerable variation across regions for a given species, decreasing in some and increasing in others. Overall, our models indicate that the worst invasive species of today may not be the worst of tomorrow.

Keywords:*climate change, invasive species, ants, ecological niche models*

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S903F03

Conservation, Biodiversity and Climate Change

Elucidating changes in biodiversity with museum collections and biodiversity informatics

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Insect collections hold millions of data that are essential to understanding trends in biodiversity: primarily specimens, taxonomic determinations, and collecting events (where, when, and how a specimen was collected and by whom). These data have traditionally been recorded in analog and mostly remain inaccessible to the broader research community. Significant recent initiatives, however, aim to free this knowledge on a large scale, using new tools and data standards. We discuss ongoing initiatives at the North Carolina State University Insect Museum, especially in the context of large-scale digitization initiatives, and make some hopeful predictions about the future of these resources. We also discuss the roles that collections have as nuclei for institutional collaboration and training and as data sources for understanding invasion biology and climate change.

Keywords:*Taxonomy, Insect Collections, Biodiversity Informatics*

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S903F04

Conservation, Biodiversity and Climate Change S903

Insect biodiversity science: Toward an integrated discipline for insects

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9.1.6. Biodiversity is a unifying concept for the totality of all living organisms included insects and human species. Insects also are the basic resource and ecological partners for sustaining our life support system. As every community is unique in species composition and environmental characteristics, its species composition and biodiversity structure is the essence of biodiversity assessment and the basis for conservation and all other site-based activities which also offers basic educational materials on local biodiversity for K-12 and beyond. Today's knowledge of biodiversity is the culmination of biodiversity research by taxonomists for over 250 years but it is small with barely 1.75 million species documented, less than 20% of 10 million currently extant, of which insects represent over one million species. Taxonomic research must continue to expand our knowledge base on biodiversity, taxonomic training needs enhanced, and taxonomic resource and services readily available for species identification and biodiversity assessment. Biodiversity science integrating taxonomy, community ecology, genomics and application science must take the center-stage for conservation of life; specifically insects that represent the largest block of biodiversity should play a major role in advancing it. In this context insect biodiversity course was developed by integrating all these subdisciplines for a broad audience. This course was first offered in 2008 as Principles of Insect Biodiversity Science at Penn State and then was introduced to the China Agricultural University in Beijing where it was offered in abridged format in 2010. This presentation will share some details of the course.

Keywords:*biodiversity, insects, integrated discipline, biodiversity science, graduate course offering*

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S903F05

Conservation, Biodiversity and Climate Change

Diversity of the insect fauna of a mangrove forest in southern Mexico from the Middle Miocene to the present

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Abstract: The present work is a comparative analysis of the fossil insect fauna from Hymenaea mexicana, the origin plant of Mexican amber, to the recent insect fauna from Bursera simaruba and Hymenaea courbaril L., the closest related tree to H. Mexicana in Central America. The collection of recent insects was carried out in a Biosphere Reserve within a Mangrove forest located at the pacific coast in the south of Mexico. This region was chosen because it is very similar to the former Mexican amber forest according to the floral composition, climate, and geographic location. The information of the fossil material was gathered from different Mexican amber collections around the world. One special focus of this work lies on the taphonomy of amber. This, to find answers to key questions concerning taphonomic biases and filters of fossilization processes from the former living fauna to the amber faunas of the Miocene Mexican amber forest. Once the taphonomic processes in amber are understood and it is recognized which information is lost and which information is remained in the fossil record, the stability of faunal communities in a Mangrove ecosystem through geological ages can be analyzed and discussed. The results reveal how similar the recent fauna associated with Hymenaea courbaril L and Bursera simaruba is to the fossil fauna of H. mexicana. In addition, the present work is a contribution to the knowledge of the insect biodiversity of a highly diverse Neotropical Region, like southern Mexico.

Keywords:*amber, taphonomy, Mexico, fossil insects, diversity*

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S903F06

Conservation, Biodiversity and Climate Change S903

Taphonomical and ecological implications of fossil and recent Psychodidae (Insecta, Diptera) from Mexico

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The present work is a comparative study of the fossil Psychodid fauna from the Miocene Mexican amber with the extant Psychodidae fauna from Southern Mexico. Today Psychodidae are distributed worldwide and are also well known from the fossil record of several amber deposits. The larvae can be found in various aquatic and terrestrial habitats. Living Psychodidae have been collected in a mangrove region of a biosphere reservat south of Chiapas with vegetation quite similar to the former Mexican amber forest. A comparison of the fossil fauna with the recent fauna provides the opportunity to study insect diversity and the stability of faunal communities over geological time. However, the fossilization in amber is characterized by certain taphonomic processes and the information preserved in amber may be biased.Trichomyia for example is the most common Psychodid genus in Mexican amber. In contrast, this genus is very rare in the collection of recent Psychodidae. This faunal difference might imply that this genus was much more abundant during the Miocene but is rather interpreted as a result of the particular habitats of this genus. Thus, the knowledge of fossilization processes is a necessary condition for interpreting the former fauna and for drawing conclusions on the stability and outlast of ecosystems. The present study directly addresses this problem of bias, loss and conservation of information during the fossilization process in amber.

Keywords:*Psychodidae, Taphonomy*

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Pheromone-based management of the red clover casebearer moth, an invasive pest of clover in Canada

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The red clover casebearer (RCC), Coleophora deauratella, is an invasive seed predator of red clover, Trifolium pratense, in Canada. Larval infestations can cause up to 99.5% seed loss. Insecticide does not significantly reduce damage caused by RCC, and the cost of application is not offset by seed yield. Lack of insecticide control options makes RCC an ideal candidate for pheromone-mediated communication disruption. Sex pheromone-based communication disruption of C. deauratella was examined by application of synthetic sex pheromone in small plot trials. Rope dispensers (Shin Etsu, Japan) and puffers (Suterra, OR, USA) formulated with C. deauratella pheromone consisting of a 10:1 ratio of Z-7-dodecenyl acetate and Z-5-dodecenyl acetate were tested in Alberta. Moth capture was reduced in rope dispenser- and pheromone puffer-treated plots as compared to non-treated control plots. Mechanisms of communication disruption were assessed by comparing the efficacy of pheromone rope dispensers containing the attractive full pheromone blend (10:1 ratio of Z-7-dodecenyl acetate and Z-5-dodecenyl acetate) or major component only (Z-7-dodecenyl acetate) in small plot trials. As well, pheromone rope dispenser density and dose was varied in order to optimize pheromone-mediated communication disruption. The methods used during these field studies can be combined into an integrated pest management program to potentially mitigate the current RCC outbreak.

Keywords:*mating disruption, pheromone, clover, Lepidoptera, Coleophora*

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S1001M03

Integrated Pest Management

Pheromone-based monitoring of the weevil pests in the tropics

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Surveys of major weevil pests (Coleoptera: Curculionidae) of the agricultural crops in the tropics have been conducted and their occurrence has been reported. Ultimately, pheromone-baited traps are developed for population monitoring studies and possibly for the control of the weevil pests. The banana root borer Cosmopolites sordidus is cosmopolitan, and it is one of the main pests occurring in banana plantations throughout the world. The New Guinea Sugarcane Weevil Rhabdocelus obscurus is an introduced pest to Guam and its neighboring islands. Similarly, sweetpotato weevil Cylas formicarius is recognized as the most important pest of sweet potato worldwide and is known to cause considerable damage, with reported losses ranging from 5-100%. All three weevils are economically important pests in the tropics. The cryptic feeding habits of the larvae and the nocturnal activity of the adults make detection and control of infestations difficult. Sex pheromones have been effective in detecting infestations at even low population levels. Consequently, consideration in recent years has focused on synthetic sex pheromones as a potential component in insect control and management of weevil pests. Pheromone-based trapping techniques have been developed by evaluating various trap types and dimensions, color and placement, and heights of the traps in the field on Guam. The results will be discussed.

Keywords:*Pheromones, monitoring, weevil pests, tropics*

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S1001M04

Integrated Pest Management

Chemically-Based Monitoring of the Brown Marmorated Stink Bug, *Halyomorpha halys* (Stal)

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The brown marmorated stink bug, Halyomorpha halys (Stal) (Hemiptera: Pentatomidae), is a is native to China, Japan, Korea, and Taiwan, but was accidently introduced into North America in the mid 1990s and has been officially detected in over 35 states in the United States and in Ontario, Canada. This insect has emerged as a pest of unprecedented importance in orchard crops, small fruit, grape, vegetables, row crops, and ornamentals in the mid-Atlantic region of the United States. Danage in apple alone resulted in losses in excess of 37 million dollars based on analysis by the U.S. Apple Association in 2010. Until recently, there was no established method to detect presence, abundance or seasonal activity of brown marmorated stink bug in any cropping system. Here we report on progress to develop a sensitive and reliable pheromone-baited monitoring trap for brown marmorated stink bug to enable growers to make more informed pest management decisions.

Keywords:*Halyomorpha halys, brown marmorated stink bug, pheromone, trapping*

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Mating disruption of multiple species in horticulture.

Alexandre Ilichev
Department of Primary Industries Victoria, Australia

Pheromone-mediated mating disruption (MD) is an important tool of sustainable, effective and environmentally-friendly Integrated Pest Management (IPM) systems in Horticulture. The successful use of hand-applied MD dispensers for long-term sustainable control of oriental fruit moth (OFM) and codling moth (CM) have been well documented for over 20 years in Australian orchards. At the beginning the general approach was to treat individual orchard blocks and only known host-plant with MD for individual pest species. The ability of OFM and CM to migrate between orchards, quickly invade new host-plants and together damage the same variety stimulated grower's need to cater for both pests in their IPM strategies. Our results of field trials conducted over three consecutive seasons demonstrated that dual-species hand-applied dispensers, designed to disrupt both CM and OFM, were as effective as single-species dispensers applied individually by reducing moth numbers and fruit damage to a similar degree. However, disruption efficacy of some dual-species dispensers was significantly lower near the end of the season for OFM because of depletion of active ingredients. Effective disruption of CM and OFM was also obtained with a multi-species formulation that releases the main pheromone components of both CM and OFM and several leafroller species. Our results provided evidence that simultaneous disruption of several Tortricidae species with a single hand-applied dispenser will be economically advantageous in regions where control of multiple pests is needed in the same variety. Some problems of simultaneous disruption of multiple species in horticultural crops will also be discussed.

Keywords:*mating disruption, oriental fruit moth, codling moth, tortricidae*

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S1001M06

Integrated Pest Management

Plant volatiles attracting an invasive fruit moth: Basic findings relevant for future monitoring

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In many agro-ecosystems, invasive insect pests threaten the carefully implemented IPM strategies. Monitoring immigrating females is highly desirable. Using the model system composed of the oriental fruit moth Grapholita (=Cydia) molesta and volatiles from its primary host peach, we addressed some basic questions of potential relevance for many cropping systems. The oriental fruit moth is an invasive fruit pest, which is still expanding its distribution range. How does this pest exploit the odor complexity of its host? We showed that female attraction relies on a synergistic interaction of two blend odor constituents, which include even quantitatively minor compounds. Furthermore, how do female moths respond to changing ratios of odor constituents in the blend? Behavioral experiments and neurophysiological studies consistently showed that the ratio specificity of the females is less narrow than previously assumed. This is a promising feature for future field application of volatile blends to assess female incidence and timing of their appearance A relatively robust response of females to changing ratios of volatile constituents might apply to other species as well, as adult females in search of suitable oviposition sites typically have to cope with changing volatile emissions from their host plant in the field. Invited submission: Chemical ecology and crop protection (10-2.)

Keywords:*Grapholita molesta, volatiles, behavior, attraction, neurophysiology*

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S1001M07

Integrated Pest Management

S1001

Use of a plant-volatile moth attractant in resistance management for transgenic cotton.

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We have recently registered a product called Magnet®, for attract-and-kill of adults of Helicoverpa armigera and H. punctigera in Australian cotton. It consists of a mixture of six synthetic (nature-equivalent) plant volatile chemicals, with sucrose as a feeding stimulant. Farmers can add any one of three insecticides. When applied to cotton crops, Magnet® with insecticide is capable of attracting and killing large numbers of moths. It was developed as a component for IPM in conventional (non-Bt) cotton, but this now occupies a very small proportion of Australian cotton acreage, which is dominated by transgenic Bt (Bollgard II ®) cotton. Recent trends in the frequency of resistant alleles for the two Cry toxins in Bollgard II ® suggest that additional tools may be needed for inclusion in future Resistance Management Plans, either to supplement existing measures or to replace onerous requirements such as cultivation to destroy overwintering pupae. In this presentation we will describe experiments aimed at enhancing the productivity of refuges which are used to generate unselected moths by application of Magnet® without insecticide, or (by including insecticide) killing moths emerging from late season cotton, or emerging in spring from fields which grew cotton in the previous summer.

Keywords:*attract-and-kill, plant volatiles, Helicoverpa, resistance management, cotton*

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S1001M08

Integrated Pest Management

"Attract and reward" as a contribution to pest management

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Introduction: Conservation biological control (CBC) can be improved by combining two 'eco-technologies' to enhance the ability of natural enemies to reduce pest populations. In the 'attract and reward' (A&R) approach, synthetic herbivore induced plant volatiles (HIPVs) (attract) are combined with floral resource supplementation (reward). As a result, enemies' ecological fitness are likely to be improved. Methods: A full two-factorial randomized block design was used in Brassicas. Each block included one treatment with a sachet of MeSA, one with flowering buckwheat, one combining the two; the control consisted of the crop alone. The first experiment targeted, three parasitoid, one hoverfly and one lacewing species and Anacharis zealandica at the the fourth trophic level. In the second, peach and cabbage aphids were targeted with their parasitoids and hyper-parasitoids. Results: No synergistic effect of 'attract' and 'reward' was observed in either experiment. Some natural enemy populations increased in response to MeSA, others to buckwheat and some were repelled. MeSA led to had significantly higher aphid parasitism rate. Conclusion Combining MeSA and buckwheat could be beneficial as the two techniques increase the abundance of different natural enemies, as long as any unwanted side effects can be managed.

Keywords:*biological control*

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S1001M09

Integrated Pest Management

Root feeders induce volatiles attracting predators

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Volatiles resulting from plant ? herbivore interactions play a major role in resource foraging by predators and parasitoids; they can be involved in orientation processes, oviposition or feeding site acceptance or patch quality estimation. In the present study we investigated the influence of dimethyl disulfide (DMDS), a major volatile constituent of Brassica napus roots heavily infested by the cabbage root fly, Delia radicum, on orientation and resource use by its main coleopteran predators. We first carried out laboratory studies where we showed that the staphylinid beetle Aleochara bilineata is strongly attracted by this compound and uses it for microhabitat choice. Attractiveness of this compound was also tested in the field, in a naturally complex odorous environment. Using an original setup, specially designed for ground dwelling beetles, different concentrations of the pure molecule as well as attractiveness of the natural blend emitted by infested roots were tested simultaneously. The main predators of D. radicum (i.e. the two staphylinids A. bilineata and A. bipustulata and carabid beetles of the genus Bembidion) were shown to be significantly attracted by DMDS but responded in different ways both to the natural blend and to the different concentrations tested. The potential resources searched by each species and their specificity for these resources could explain the differences in the responses of the 3 ground-dwelling predators. The possible use of DMDS for enhancing the natural control of D. radicum, a major pest of cabbage crops in northern Europe, will be discussed.

Keywords:*Cabbage root fly, ground dwelling beetles, resource foraging, biological control*

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S1002M01

Integrated Pest Management

The distribution and host range of SWD from the Eastern Asia with development of diagnostic method using DNA barcode

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Spotted wing drosophila (SWD) or cherry drosophila, Drosophila suzukii Matsumura, a native of Asia, is a pest of small and stone fruits. This species was first observed in mainland Japan in 1916 and described by Matsumura in 1931. It is a species widely distributed in South Korea and Japan. Although there have been records of causing damage to Korean-type cherries (Prunus tomentosa) within South Korea in 1935, no reports of damages done to any crops have been reported since. Therefore, this species has not been considered as a pest insect, and no studies have been conducted excluding some faunistic survey. In Japan, more studies both taxonomic and ecological, concerning the SWD have been extensively researched. We installed clear plastic bottle traps containing apple cider vinegar (ACV) and wine in 20 sites (mountains, vineyards, table grape packing houses, blueberry orchards, strawberry farms) within Korea, examining the state of SWD population fluctuation from last September. In addition, we conducted a DNA barcode analysis to identify immature stage of SWD. In this symposium, the following subjects will be introduced: first, the state of distribution and host ranges of SWD in Korea and Japan; second, the introduction of research conducted in Korea; third, the identification technique of SWD based on molecular characteristics.

Keywords:*SWD, Drosophila suzukii, distribution, host range, Korea, Japan, DNA barcode*

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Drosophila suzukii (Diptera: Drosophilidae) in Europe: Geographic distribution, biology and economic impact three years after detection.

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Unlike other Drosophila species, Drosophila suzukii (Matsumura) is economically important as enabling to infest fruits before harvest. In 2009 it was detected for the first time in Italy on raspberry, blueberry and strawberry. The EPPO included D. suzukii in the Pest Alert List; and in 2010 a Pest Risk Analysis was performed as it was considered a threat to all European countries. Extensive monitoring in 2010-2011 in the Italian regions as well as in other European countries confirmed its high dispersal rate and capability to infest a wide range of cultivated and spontaneous hosts. Significant attacks of D. suzukii on some thin skin cultivars of grape was reported in the Northern Italian regions in fall 2011 though not causing economic losses. Three years after the first detection D. suzukii has been reported in 9 countries (Italy, France, Spain, Switzerland, Austria, Slovenia, Croatia, Germany, Belgium). Despite the severe chemical control, important crop losses were found on berry fruits and cherry due to high insect population density and to the patched distribution of the crops. The increasing number of insecticide applications jeopardizes the results obtained with IPM, therefore development of alternative control methods is urgent for an economic safeguard of the concerned fruit industry. Mass trapping and physical crop protection by using anti-insect nets are under experimental evaluation and seems the more promising alternative control strategies accessible in the near future. Improvement of the attraction efficiency of the available lure is the major objective of the research teams dealing with chemical ecology.

Keywords:*IPM, alternative control methods, chemical ecology, anti-insect net, mass trapping*

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S1002M03

Integrated Pest Management

S1002

Spotted wing *Drosophila*, a new berry and cherry pest in the Western USA

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The spotted wing drosophila, Drosophila suzukii (Diptera: Drosophilidae) was first found in California, USA in 2008 and quickly spread north into Oregon and Washington by 2009. Originally from Asia, D. suzukii is now impacting berry and cherry fruits on both coasts of the USA as well as being found in parts of Europe. This pest is problematic in that it attacks ripening fruit and additional sprays are usually required to control it. Field and laboratory studies indicate that pyrethroids, spinosad, spinetoram and organophosphorus insecticides are the most effective insecticides against this pest. One result of this introduced pest is that integrated pest management (IPM) programs are being negatively impacted because of the need for additional applications of insecticides.

Keywords:*Drosophila suzukii, IPM, insecticide, stone fruit, berries*

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S1002M04

Integrated Pest Management

S1002

Status of spotted wing fruit fly, *Drosophila suzukii*, in China

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The Drosophila melanogaster species group in China comprises at least 67 species. Among them, spotted wing fruit fly, Drosophila suzukii Matsumura, is one the six most widespread species, and it is found in at least 28 provinces. The infestation status of the pest in recent years and its control techniques in China will be presented, as well as future research directions.

Keywords:*Drosophila suzukii, China, Pest status, Distribution*

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S1002M05

Integrated Pest Management

Drosophila suzukii host selection and utilization in eastern United States agroecosystems

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Drosophila suzukii (spotted wing drosophila, SWD) is a devastating pest of soft skinned fruit that was first detected in the southeastern United States in Fall 2009,following an initial detection in North America in 2008. It has since spread rapidly throughout the eastern US, making it a national pest management issue. Significant SWD damage has occurred in caneberries, blueberries, and grapes throughout the eastern US during the summer of 2011. SWD biology, ecology, and management appears to differ between the eastern and western US and within the eastern US. Low infestation rates and little economic damage have been reported in Florida, while significant infestations and crop losses have occurred elsewhere. Eastern US farmscapes present SWD with a range of crops that have overlapping fruit, and a diversity of native host plants with ripening fruit throughout the summer and fall, providing refuges outside crop fields. Crop infestation rates have been highest in raspberries, and field and laboratory experiments suggest that host species, host variety, and substrate firmness influence both SWD host selection and offspring performance. The implications of host selection and offspring performance are being assessed under field conditions throughout the eastern US and will aid in assessing risk of SWD damage and in making management recommendations.

Keywords:*invasive species, IPM, host preference, performance*

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S1002M06

Integrated Pest Management

Seasonal biology of spotted wing drosophila (*Drosophila suzukii*) in California raspberries

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First reported in California in August 2008, spotted wing drosophila, Drosophila suzukii Matsumura (Diptera: Drosophilidae), is a native of Southeast Asia and a pest of berry and stone fruit crops. Now found in much of North America, D. suzukii is a key pest for raspberry production. Drosophila suzukii pose a particularly strong threat to organic growers, as there are fewer control tactics available. To determine the seasonal population biology of Drosophila suzukii adult flies were monitored weekly via lure-based traps. Field sites were chose to cover the range of raspberry ripening phenology (early, mid, and late ripening), with both an organic and a conventionally managed raspberry site. Apple cider vinegar and yeast + sugar + water lures were used to compare attractiveness, and monthly vacuum samples were taken to ascertain potential sex-ratio bias of the lures. When ripe fruit were available, fruit infestation was measured as average number of larvae per fruit. As might be expected, numbers of adult D. suzukii trapped in each variety begin to increase as the fruit ripened, with fewer flies at the conventional site. Populations build throughout the season and drop off mid-winter. Attractiveness of the lures appears to vary by season. Although the sex ratio of trapped flies does appear to cycle, it does not appear to be an artifact of lure-based trapping. Interestingly, larvae appear in fruit before the first captures of adult flies in the traps. This highlights the need for a more attractive early season trap and knowledge of overwintering behaviors.

Keywords:*Drosophila suzukii, Monitoring*

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S1002M07

Integrated Pest Management

Insecticide Resistance Monitoring in *Drosophila suzukii*

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Drosophila suzukii (Diptera: Drosophilidae) has become a major pest of cherry and berry crops in North America with current management largely dependent on insecticide applications. Under heavy insecticide selection pressure for last two years and a generation time that may take as few as two weeks under optimal conditions, an ideal situation has been created for the development of resistance. Drosophila suzukii is primarily treated with organophosphate, pyrethroid and spinosyn insecticides in conventional production, but there also exists a particular challenge for organic growers who have fewer and generally less residual options. Fortuitously, D. suzukii is closely related to the intensively studied model organism, Drosophila melanogaster, the organism most frequently used to identify molecular target sites for resistance. Field populations of D. suzukii have been collected from California, Oregon, and Washington for the last two field seasons, then subjected to contact bioassays in a potter tower. A subsample of each of these collections has been preserved for genomic analysis, specifically targeting loci known to confer resistance in D. melanogaster. This has been coupled with a laboratory selection experiment that will attempt to quantify the impact pesticide rotation has on delaying the development of resistance. Preliminary data suggests that fly populations collected from certain California plantations are more tolerant to insecticides. D. suzukii provides a unique opportunity to compare and contrast insecticide resistance in both laboratory and field experiments via contact bioassay and genomic techniques.

Keywords:*Insecticide Resistance*

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S1002M08

Integrated Pest Management

Development of monitoring and control techniques against spotted wing Drosophila, *D. suzukii*

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Spotted wing Drosophila, D. suzukii (SWD), is a serious invasive pest in Europe. A quick dispersal in Europe together with significant damage in fruit and berries calls for instant action. Three to four years after its introduction to Spain, SWD has become the most important pest insect in fruit growing areas in in Italy, France and Spain. Unlike the ordinary fruit fly, D. melanogaster, SWD oviposits on unripe, but fully grown fruit. Shortly before harvest, spraying with insecticides conflicts with preharvest intervals. In addition, the efficacy of insecticde sprays is not satisfactory. Behaviour-modifying chemicals, pheromones and plant volatile compounds, are widely used for insect monitoring and detection and for population control. The fruit fly D. melanogaster is a model, also for research on the olfactory system. It is straightforward to apply the knowledge of the D. melanogaster olfactory system to its sister species D. suzukii. A first research project on SWD has just been started up, including field studies in Italy and USA. The goal of our research is to develop a monitoring lure and a control technique. A first account of ongoing research will be presented.

Keywords:*Drosophila suzukii, monitoring, detection, attraction, semiochemicals*

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S1002M09

Integrated Pest Management

S1002

Control of spotted wing drosophila (*Drosophila suzukii*) in California cherries

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The efficacy and residuality of all major insecticide classes for control of spotted wing drosophila will be discussed from field evaluations conducted in California cherry orchards. Timing of chemical control and new control methodology that circumvents many phytotoxicity and residue issues associated with chemical control of spotted wing drosophila will also be reported.

Keywords:*Spotted Wing Drosophila, Management, Cherry, Control*

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S1002M10

Integrated Pest Management

Determining biological parameters of spotted wing drosophila

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Basic biological parameters of a United States culture of field-collected Spotted Wing Drosophila, Drosophila suzukii, including cold tolerance, temperature-related development, and age-related oviposition and fecundity will be presented. These data were obtained from flies reared on commercially produced fruit and include cherries and blueberries. These data will be compared with data from previous studies conducted on field-collected fly cultures reared on cherries in Japan. We will consider the impacts of humidity on longevity and fecundity of adult female flies. In addition, we will discuss the survival and fecundity of flies of late-dormant survival of overwintering flies provided with nectar and pollen from cherry and blueberry blossoms. The suitability and susceptibility of different commercial fruit at increasing ripeness levels will be presented. Field scouting results from commercial farms will be discussed and directly related to the parameters described above.

Keywords:*Spotted Wing Drosophila, Drosophila suzukii, temperature related development, biological parameters*

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S1003M01

Integrated Pest Management

CyazypyrTM, a new tool against emerging and old potato insect pests around the globe

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Cyazypyr™ (DPX-HGW86, cyantraniliprole), is a novel cross-spectrum second generation anthranilic diamide insecticide that was discovered by the DuPont Company, and has proven to be effective at controlling economic insect pests in potato and many other crops. With the over abundance of neonicotinoid insecticides registered in potatoes in the US and other countries, an effective insecticide in a new class is most needed. Cyazypyr™ is the second molecule in the anthranilic diamide class of chemistry to be commercialized, but is the first with significant efficacy on sucking pests. It exhibits a novel mode of action, by selectively activating the ryanodine receptor in insect muscles, resulting in rapid cessation of feeding in affected pest insects. This causes reductions in direct feeding damage and also in the capability of the affected pest insects to vector plant diseases. Field and laboratory testing around the world has shown excellent activity of Cyazypyr™ on most old economically important potato pests and also in the new emerging pests. Cyazypyr™ presents a favorable eco-toxicological and environmental fate profile and also has very low mammalian toxicity. The Environmental Protection Agency in the USA recently granted Cyazypyr™ the Reduced Risk status. Because it conserves many beneficial arthropods, Cyazypyr ™ will be an excellent fit in IPM programs designed for insect pests in potatoes and multiple other crops.

Keywords:*Colorado potato beetle, potato tuberworm, green peach aphid, potato tomato psyllid, zebra chip*

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S1003M02

Integrated Pest Management

Alternate host induced complexity and vector-virus interactions in potato pathosystem

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Weed hosts serve as reservoirs of aphids and aphid-transmitted viruses in potato (Solanum tuberosum L.) ecosystems worldwide. The Pacific Northwest potato ecosystem is characterized by one such solanaceous weed host, hairy nightshade, Solanum sarrachoides (Sendtner). Experiments indicated that alternate hosts such as S. sarrachoides could significantly improve the fitness of colonizing aphids including the green peach aphid, Myzus persicae (Sulzer) and the potato aphid, Macrosiphum euphorbiae (Thomas). Both aphids performed better on S. sarrachoides than on potato. These effects were further enhanced when S. sarrachoides plants were infected with a Luteovirus, Potato leafroll virus (PLRV). Besides biology, the settling patterns of aphids were also influenced by S. sarrachoides and PLRV infection. Aphids preferred to settle on S. sarrachoides leaflets when compared with potato, and also on PLRV-infected plants than on non-infected plants. In addition, experiments revealed the presence of behavior modifying cues such as visual and olfactory cues that could affect aphid settling. Volatile organic compounds potentially mediating such effects were identified. Further, field studies were conducted to assess the role of these weed hosts on the spatial and temporal spread of PLRV by establishing PLRV inoculum foci. The results revealed that the presence of S. sarrachoides in the vicinity could significantly hasten the primary and secondary spread of PLRV. Observations also indicated that there could be host-specific biotypes that specialize on alternate hosts despite their polyphagous feeding abilities. The implications of the presence of such biotypes on potato viral epidemiology will be discussed.

Keywords:*Weeds, Alternate hosts, Aphididae, Solanum spp., Viral epidemics*

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S1003M03

Integrated Pest Management

The potato tuberworm in the USA : An exotic or invasive pest?

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The potato tuberworm (PTW), Phthorimaea operculella (Zeller), is by far one of the most important constraints to potato productivity worldwide. In the USA, PTW has been reported in California, Arizona, New Mexico, Texas, Utah, as far east as in Maryland and Virginia, Oregon, Washington and Idaho. Control of PTW damage is critical because its larvae infest tubers (in the field as well as in storage), rendering tubers unmarketable. There is a zero level of tolerance in processed potatoes. Determining the origin of PTW populations will help us understand how they were introduced and how to prevent further introductions.

Keywords:*potato, potato tuberworm, IPM, insects, potato tubermoth. Phthorimaea operculella*

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S1003M04

Integrated Pest Management

Colorado Potato Beetle: Why is it such a pain in the neck?

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University of Maine, U.S.A.

The Colorado potato beetle is a very important defoliator of potatoes in many potato-growing areas of the world, and it continues to expand its geographic range. Although this insect has received enormous attention from the scientific community, it remains a formidable threat to the potato industry. The beetle has a diverse and flexible life history that is well-suited to a variety of environments. It is also extremely adaptable to adverse conditions, including those created by humans in an attempt to control the pest. The beetles integrate diapause, dispersal, feeding, and reproduction into an ecological 'bet-hedging' strategy, distributing their offspring in both space (within and between host habitats) and time (within and between seasons). As a result, they are a very challenging pest to manage. Unfortunately, there will most likely be no "silver bullet" solution to the problem created by this insect. The only sustainable way to protect potato crops is to integrate multiple control techniques into a scientifically sound management approach. This is not an easy task, but the only alternatives are recurrent crop losses in combination with environmental degradation.

Keywords:*Leptinotarsa decemlineata, Solanum tuberosum, life history, evolutionary plasticity, IPM*

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S1003M05

Integrated Pest Management

Same diet but different exploitation strategies; an aphid tale.

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Phloemophagous insects have the ability to fine tune plant physiology to exploit their sap diet keeping phloem cells alive and avoiding the clogging of the sieve tubes to maintain sap fluxes. However their interaction with their host plant can in turn alter plant quality. We showed that despite a identical mode of feeding and similar nutritional requirements, potato systemic response to the sympatric aphids Macrosiphum euphorbiae and Myzus persicae varied according to the infesting aphid species. Monitoring aphid feeding behavior using EPG technique revealed that i) heterospecific preinfestation led to reduced plant acceptance only when M. euphorbiae was the preinfesting species ii) in case of conspecific preinfestation both species induced reduced plant acceptance and ii) this alteration of the aphid feeding behavior was due to the expression of a phloem located antixenosis. To start investigating the mode of plant manipulation by these two aphids, we developed a proteomic approach and showed that protein patterns of potato phloem sap strongly varied depending on the species of the infesting aphid. Different proteins, in particular a NBS-LRR one, are specifically induced or repressed in response to one or the other aphid. Taken together, these results suggest that much more than M. euphorbiae, M. persicae needs to deeply manipulate plant physiology to ensure a compatible interaction with the plant. On the one hand the long lasting coevolving between M. euphorbiae and the Solanum species, on the other hand the larger host plant spectrum exploited by M. persicae could explain such variations in potato response.

Keywords:*aphid, feeding behavior, plant response, phloem proteome*

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The potato virus Y tale: Studying the aphid vectors and preventing transmission

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Potato virus Y (PVY) (Potyviridae; Potyvirus) is one of the most widely distributed pathogens in the potato crop worldwide, causing significant yield reductions and quality degradations in potatoes. It is transmitted in a non-persistent manner by several species of aphids. New molecular tools are now available to identify PVY vectors in the field. Host selection behaviour on potato is modulating the efficiency with which a given species of aphid is transmitting the virus. Mineral oil is the only effective way to inhibit transmission. The concentration and location in the plant of mineral oil are critical to observe effective protection of the potato crop.

Keywords:*Potato virus Y, aphids, behaviour, mineral oil*

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S1003M07

Integrated Pest Management

The impacts of soybean aphid, a recent invasive to North America, on seed potato production

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Prior to the arrival of the soybean aphid, Aphis glycines Matsumura, the primary aphids caught in Midwest potato fields were grain aphids and the turnip aphid, Lipaphis pseudobrassicae (Davis). Soybean aphid now makes up more than 47% of aphids caught in potato fields. Thus, our research focused on soybean aphid and the impact its arrival was having on potato seed production. Soybean aphid is a capable Potato virus Y (PVY) vector (33% vector efficiency), transmitting all PVY strains equally. Soybean aphid is also a vector of Potato leafroll virus (PLRV) (7% vector efficiency) and when in high populations, can significantly influence field spread of PLRV. Soybean aphid does not colonize potato but does land in potato fields with two distinct peaks in the growing season; early June and late August. Electrical penetration graph studies indicate soybean aphid probes just as often and as long as green peach aphid, Myzus persicae (Sulzer). Since few potato seed lots are entirely PVY-free when planted, cultural techniques to limit virus spread such as crop borders and mineral oils are now less effective due to soybean aphid. The failure of potato seed certification programs to end the PVY epidemic and the fact that cultural practices that have been effective in the past are no longer able to control PVY spread, clearly indicates the impact this invasive virus vector has had on North American seed potato production.

Keywords:*soybean aphid, PVY, potato, PLRV*

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The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake

Johan Coert Vanlenteren
Wageningen University, Netherlands

In commercial augmentative biological control, natural enemies are mass-reared in biofactories for release in large numbers to obtain an immediate control of pests. The history of commercial mass production of natural enemies spans a period of roughly 120 years. It has been a successful, environmentally and economically sound alternative for chemical pest control in crops like fruit orchards, maize, cotton, sugar cane, soybean, vineyards and greenhouses. Currently, augmentative biological control is in a critical phase, even though during the past decades it has moved from a cottage industry to professional production. Many efficient natural enemies have been discovered and more than 230 species are commercially available today. Natural enemy producers developed quality control guidelines, mass production, shipment and release methods as well as adequate guidance for farmers. However, augmentative biological control is applied on a frustratingly small acreage. I will review trends in research and application, causes explaining the limited uptake and ways to increase application of augmentative biological control.Ref: Lenteren, J.C. van, 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57: 71-84. DOI: 10.1007/s10526-011-9395-1.
Free download of paper at: http://www.springerlink.com/content/b3r301232kr7251h/fulltext.pdf

Keywords:*augmentative biological control, natural enemies, failure of uptake, bright future*

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Biological control of *Tuta absoluta* by new species of Heteropteran predators

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The tomato leafminer Tuta absoluta is native to western South America, but is now an extremely devastating pest in tomato crops in most of South America, Europe and Africa North of the Sahel. This pest can cause yield losses up to 100% and decrease of fruit quality in open field and greenhouse crops. In addition to this pest, in Brazil two other lepidopteran pests - Neoleucinodes elegantalis and Helicoverpa zea - are very important on tomato. For control of the three pests, frequent applications of pesticides of up to 5 times per week are needed, and these resulted in the appearance of resistant populations to a number of active ingredients and the elimination of natural enemies. Biological control may offer a better and safer opportunity for control of these pests. Mirid predatory bugs are currently used with success in Europe to control T. absoluta. In Brazil, three newly found mirid species are now under evaluation. In the evaluation process, two questions emerged: (1) what might be the best type of natural enemy for control of T. absoluta and the other lepidopteran pests: predators or parasitoids, and (2) what characteristics should first be studied to identify an efficient predator of T. absoluta? Recent work on the new Brazilian predators will be presented and the evaluation process will be discussed.

Keywords:*predatory bug, tomato damage, predatory capacity*

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Influence of natural and supplemental lighting on predation and oviposition by predatory mites on western flower thrips

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Lighting conditions in the greenhouse vary substantially with the season. In addition, supplemental lighting is becoming more common in northern temperate greenhouses in order to provide year round production of greenhouse vegetables. Anecdotel evidence suggests that the performance of the predatory mites, Amblyseius swirskii and Neoseiulus cucumeris differs between summer and winter climatic conditions. Both species provide effective biological control of western flower thrips during the summer period, but control of thrips can be much more difficult during the winter season. Earlier studies for N. cucumeris indicate that light intensity or photoperiod did not influence its predation rate, but, perhaps, lighting conditions did impact its oviposition rate. In the past, temperature has been shown to influence the predation rate of N. cucumeris. However, oviposition was not significantly affected. Also, very little information is known about the influence of supplemental lighting on the control efficiency by predatory mites. For supplemental lighting, light emitting diodes (LEDs) and high pressure sodium lamps are the predominate light sources. The current study examines the influence of seasonal natural lighting, LED lighting and temperature on the predation and oviposition rates of N. cucumeris and A. swirskii when offered first instar western flower thrips as the only food source and its impact on these mites as biological control agents.

Keywords:*seasonal lighting, supplemental lighting, predation and oviposition rates, A. swirskii, N. cucumeris*

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The effect of photoperiod and light quality on biology of *Macrolophus pygmaeus*, a predator of whiteflies

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Fecundity, longevity and egg hatching of the predatory bug Macrolophus pygmaeus was studied in three experiments under artificial lighting in growth chambers to assess if light quality and photoperiod affect its poor establishment in winter-time tomato crops at northern latitudes. In the first experiment, the effect of photoperiod was studied using fluorescent bulbs (FLs) at 16L:8D, 13L:11D, 11L:13D, and 8L:16D at 180 µmol m-2s-1. In the second, the effect of light quality was assessed by producing the 16L:8D photoperiod with FLs, HPSLs and HPSLs supplemented with red LEDs (11 + 5h). In the third, 16L:8D, 11L:13D, 8L:16D photoperiods were produced by HPSLs and 16L:8D with FLs. In all treatments, females (feeding on Ephestia kuehniella eggs on tobacco leaf disks) oviposited and the eggs hatched: no reproductive diapause was induced. Fecundity increased with decreasing photoperiod irrespective of light quality. In FLs, fecundity was significantly lower than in HPSLs alone or in HPSLs + red LEDs in one experiment, whereas no difference was found between FLs and HPSLs in the other experiment. The results suggest that HPSLs do not adversely affect reproductive biology of the bug, and that part of the long photoperiod could be replaced with red LEDs without negatively affecting the bug's biology. The 16-hour photoperiod produced by artificial lighting in year-round tomato production, however, is not optimal for the fecundity of M. pygmaeus. Further research is needed from greenhouse winter conditions where the bugs perceive also the natural light quality and short photoperiod.

Keywords:*Macrolophus pygmaeus, photoperiod, light quality, biological control, whiteflies*

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Impact of secondary parasitism on *Aphidius colemani* in the banker plant system on aphid control in commercial greenhouses in Japan

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In order to elucidate the impact of secondary parasitism on a banker plant system, the aphid parasitoids on banker plants were surveyed for four years at commercial greenhouses producing eggplant and sweet pepper in Kochi Prefecture, Japan. A banker plant system using Aphidius colemani was introduced into the greenhouses in November or December for the control of pest aphids from February through May. The mean ratios of secondary parasitoids to the total parasitoids on the alternative host aphid (Rhopalosiphum padi) on banker plants were less than 35% in the early season. In March or April, the mean ratios exceeded 40%, and they reached more than 70% by May or June. Mainly, three species of secondary parasitoids, Alloxysta sp. nr victrix, Dendrocerus laticeps and Syrphophagus sp., occurred through the season. In addition, Asaphes suspensus and Pachynouron aphidis occurred in the later season. In March and April, the higher ratios of secondary parasitism on banker plants had a significant influence on the failures in aphid control during the main harvesting season. On crop plants, higher ratios of secondary parasitism in any season tend to induce failure in aphid control. Though such a negative influence has been suggested theoretically, and has also been supported through some trials of the banker plant system in small greenhouses, no study has heretofore demonstrated the relationship between secondary parasitism and aphid-control results in commercial greenhouses using the banker plant system.

Keywords:*banker plant system, Aphidius colemani, secondary parasitoid, commercial greenhouse, aphid control*

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Development of a banker plant system for *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) to control aphids on sweet pepper from summer to autumn in Japan.

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A banker plant system has been used to control pest aphids, mainly on solanaceous crops in greenhouses, from autumn to spring in Japan. The system consists of barley as banker plant, Rhopalosiphum padi (L.) as alternative host, and Aphidius colemani Vierek as a natural enemy. However, use of this banker plant system becomes impractical under high temperature conditions from late spring to early autumn because of the poor growth of barley plants. In addition, the outbreak of hyperparasitoids of A. colemani causes a severe reduction of the control effect of the banker plant system, especially in the hot season. To avoid such problems, a new banker plant system using the aphidophagous gall midge, Aphidoletes aphidimyza (Rondani), is being developed as an alternative method. To establish such a system using A. aphidimyza, the reproduction of four cereal aphid species, Melanaphis sacchari (Zehntner), R. maidis (Fitch), R. padi (L.), and Schizaphis graminum (Rondani), reared on sorghum or barley, was compared under four constant temperatures. The results showed that the reproduction rate of M. sacchari, reared on sorghum, was the highest among all combinations at high temperature. The efficiency of the banker plant system using sorghum seedlings, M. sacchari, and A. aphidimyza in controlling aphids on greenhouse sweet peppers was evaluated in Maizuru City, Kyoto Prefecture. The density, and the frequency of occurrence, of pest aphid on the sweet pepper plants were suppressed more effectively in the greenhouses with the sorghum banker system than in those with barley banker system.

Keywords:*banker plant system, Aphidoletes aphidimyza, Melanaphis sacchari, sorghum, sweet pepper*

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Competitive interactions between aphelinid parasitoids and the implications for population stability and biological control applications

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In classical biological control, introducing single or multiple natural enemy species to reduce the density of an invasive pest to a tolerable level is the longtime debate. Bemisia tabaci (Gennadius) biotype B is the most serious invasive pest in China. To manage this superbug, two promising parasitoid species of whitefly, Eretmocerus hayati (Rose and Zolnerowich) and Encarsia sophia (Girault), were introduced into China in 2008. To better understand the consequence on pest suppression by competitive interactions between natural enemies. First, their ecological suitability, including the temperature, host plant and host instar suitability, as well as the shifting host handling strategy under varying host densities were investigated. Subsequently, the host discrimination ability of parasitoid females on conspecific and heterospecific parasitized hosts, as well as the handling efficacy on such parasitized or healthy hosts was measured. The results suggesting that at low host density, when the accepted host instars range overlapped, this two species may interfer to one another. To reveal whether the interference between this two parasitoids reduce the biological control efficacy, we tested the consepecific and the heterospecific interference competition including hyperparasitizing, multiparasitizing and host-feeding on parasitized hosts. Finally, a field cage release experiment was conducted. The results coincided with the laboratory competition experiment, that two parasitoid species coexisted through a balance between the competitive advantage afforded by interference competition and the primary predator or parasitoid's greater efficiency at attacking the pest, and the control effect of combined release was higher than that of single species release.

Keywords:*Bemisia tabaci, Eretmocerus hayati, Encarsia sophia, biological control, interference competition*

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Bee vectoring technology for crop protection and recent studies in Korea

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Bee-vectoring technology uses the capacity of bees that can carry fungal spores, bacteria and viruses as well as pollens, to deliver microbial control agents for crop protection purposes. Microbial control agents for controlling plant diseases and insect pests are delivered to flowers or leaves of plants during the pollination activities of bees. Both successful crop protection and pollination can be achieved through the manipulation of this technology. Since the early 1990s, this technology has been developed and has showed good potential for crop protection. However, the adoption of this technology at farmer's fields is still low. The aim of this study was to enhance the practical application of this technology. Three components are important to the successful application of bee vectoring: microbial control agents, dispenser design, and safety to the vector. Through the screen cage experiments using a Bacillus thuringiensis (Bt) product for the last three years, we could collect detectable densities of Bt from the flowers and leaves of sweet pepper and confirm Spodoptera litura control effect using this technology. We also designed a new dispenser for the bumble bee, Bombus terrestris, to improve their activities and delivery rate of microbials. The effects of several commercial microbial agents (Bt aizawai, Bt kurstaki and Bacillus subtilis) on the safety of bumble bees were tested through the cage trials, and some preliminary observations in greenhouse tomatoes were also done. Our studies have shown that it is critical to review these key components for feasibility before field application for the bee-vectoring technology.

Keywords:*Bee vectoring technology, Crop Protection*

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Evaluation of integrated and biological control of a new virus disease, *cucurbit chlorotic yellows virus* (CCYV), transmitted by whiteflies, using a simple mathematical model

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The cucurbit chlorotic yellows virus (CCYV) is a new virus which was first recognised in Japan in 2007. This virus is transmitted by both type B and Q of Bemisia tabaci and cause serious damage on cucurbits in greenhouses. Bemisia tabaci easily develop resistance to insecticides if farmers only rely on application of chemicals. IPM system has to be developed to suppress this virus disease. A difference equation model has been developed to predict infection of melon plants by CCYV in a greenhouse. The model can predict the number of infected plants and whiteflies every 10 days and calculation using the model is easily performed by Excel 2007. It is assumed that a whitefly adult becomes viruliferous after inoculating infected plants for 10 days and loses its ability of transmitting virus if it does not suck fluids from infected plants for 10 days. The latent period of infected plants until development of the first symptom of the virus disease is assumed to be 20 days. Other parameters of the model are the reproductive rate of whiteflies and the number of plants which one viruliferous whitefly adult can transmit the virus in 10 days. It was predicted from the model that all plants are infected 40 days after the first appearance of the symptom of the virus disease. The effectiveness of biological control, screening, use of resistant varieties for suppressing the disease is evaluated using the model.

Keywords:*whitefly, virus disease, CCYV, IPM, model*

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Developing IPM for artificially lighted year-round crops: An activity theoretical approach

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Change Laboratory, a method to help practitioners to develop working practices, is presented here as a tool for supporting collaborative transformation of the innovation process of pest management. The tool was initially used in Finnish Ostrobothnia greenhouse cluster to develop collective management strategies against the greenhouse whitefly in the current conditions and in anticipation of the potential invasion of Bemisia tabaci. We present how the whitefly problem and its ramifications were analysed and modelled in the Change Laboratory process, and introduce the initial solution concept that was developed in collaboration by growers, local advisors and researchers. The basic models of the solution concept are pest monitoring and knowledge sharing between growers as well as learning from each others' pest management actions. Furthermore, spreading of these activities from the initial study site into other villages in the area in the form of a collaborative study and problem solving group that is by the local advisors was an expansion of the initial solution concept. Based on the problem analysis and solution concept the research collective focused itsfirst research efforts on developing economic thresholds and sampling designs to improve and economize monitoring. The process and its results were analyzed in terms of how the participants' agency and learning evolved in the process, how the process deviated from the planned one, and how the object of activity, i.e. the whitefly and its management, of the interacting key activity systems (i.e. greenhouse companies) was shaped in the process.

Keywords:*Trialeurodes vaporariorum, Bemisia tabaci, change laboratory, activity theory*

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Integration of biological and chemical control in greenhouses in California: A brief history and future directions

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California's floriculture had a value of $1.01 billion in sales, comprising 25.4 percent of the United States total wholesale value. Cut flower production in California is particularly strong, accounting for about 77.3 percent of the total cut flower wholesale value in the country. Grown for its aesthetics, floricultural crops have been heavy users of traditional pesticides, and economic thresholds were virtually non-existent. Today growers recognize that spraying repeatedly does not provide 100% control and leads to resistance problems. Thus more and more growers are turning to the use of 'reduced risk' pesticides and biological control. Greenhouse whiteflies (Trialuerodes vaporarium),two-spotted spider (Tetranychus urticae), Western Flower Thrips (Frankliniella occidentalis), and the serpentine leafminer(Liriomyza trifolii) are still the major pests of many floriculture crops and are the targets for both reduced risk pesticides and biological control. Ongoing research will be reviewed that is focusing on the integration of newer botanical and insect growth regulator insecticides with: Phytoseiulus persimilis/Amblyseius californicus for spider mite control; Encarsia formosa for whitefly control; Hypoaspis sp./A. swirksii for thrips control; and Diglyphus begini for leafminer control. The commercial availability of natural enemies makes this possible at the grower level, although many are questioning the sustainability of these integrated programs due to cost. Overall, these programs have been successfully adopted in California. Pesticide use in greenhouse floriculture has declined from 95,000 to 49,000 pounds active ingredient from 2001 to 2010, respectively; even though acreage devoted to this crop has increased by more than 15% during this time.

Keywords:*Integration of Biological and Chemical Control, Greenhouse*

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Integrated Pest Management

Insect life tables: Theory, data analyses, and their applications

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Survival, stage differentiation, and reproduction are the critical elements of insect populations and are capable of revealing the population's fitness. Because the traditional female age-specific life table ignores the stage differentiation and male population, its application is limited. The age-stage, two-sex life table can take the variable developmental rates among individuals and both sexes into consideration; it can precisely describe the survival, stage differentiation, and reproduction. When reporting an age-stage, two-sex life table, the following information should be included: 1. The initial size of cohort (N), 2. The number of emerged females (Nf), 3. The age-stage survival rate sxj, 4. The age-specific survival rate (lx) and fecundity (mx). 5. The preadult mortality. Demonstrated by mathematical proof, the jackknife technique should not be used to estimate the variance of population parameters. Age-stage consumption of an insect population is important in determining the economic threshold of pests and the predation capacity of predators. To compare the potential of predators as biological control agent, we propose using the finite predation rate to include the age-stage predation rate, the population structure, and the growth rate of the predator. Computer simulation based on an age-stage, two-sex life table is capable of revealing the change of stage structure and the consumption of the pest population, as well as the interaction between the predator and prey populations. Therefore, it is a useful tool in accessing EIL, timing the release and determining the number of predators required for biological control, etc.

Keywords:*life table, theory, data analysis, computer simulation*

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Population-level effects of two acaricides and their mixture on *Tetranychus urticae* (Acari: Tetranychidae)

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Demographic toxicology based on life table analysis is suggested as the best way to study the effects of toxicants. In this study, the effects of the two acaricides, fenpyroximate , propargite, and their mixture on life history traits and life table parameters of Tetranychus urticae Koch are compared. Female adults were treated with the LC50 concentration. The age-stage, two-sex life table theory was used for data analysis. Life table parameters were calculated using the bootstrap method. Among the tested acaricides, the lowest values of the intrinsic rate of increase (r), finite rate of increase (λ) and net reproductive rate (R0) were seen in treatment with fenpyroximate (0.0913 d-1, 1.0957 d-1 and 2.8875 offspring, respectively) and the highest values belonged to those treated with propargite (0.1174 d-1, 1.1246 d-1 and 4.1861 offspring, respectively). The generation times (T) were 11.4714, 12.1347 and 12.3720 d for mites treated with fenpyroximate, propargite and the mixture, respectively. Treatment with fenpyroximate caused the shortest adult longevity of 2.55 d compared to the 4.19 d of propargite and the 3.96 d of the mixture. Fenpyroximate also led to the lowest mean fecundity of 5.96 offspring compared to propargite (7.15 offspring) and the mixture (8.46 offspring). Population projections revealed the differences in stage structure among treatments.

Keywords:*Demographic toxicology, Fenpyroximate, Life table, Propargite, Tetranychus urticae*

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Performance of dusky-veined walnut aphid, *Panaphis juglandis* (Goeze) (Hemiptera: Callaphididae), on different walnut cultivars

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Population growth parameters of dusky-veined walnut aphid, Panaphis juglandis (Goeze), were evaluated on five cultivars of walnut (Akca 1, Chandler, Fernette, Fernor, and Pedro) under field conditions in the Van region of Turkey in 2011. Experiments were carried out using potted walnut plants and plexiglas clip-cells (20 x 10 mm) with their upper side covered by muslin. Developmental time, survival and fecundity data were analyzed based on the age-stage, two-sex life table to take the variable developmental rate among individuals into consideration. The population growth rate of P. juglandis was significantly affected by the host plants. Due to longer immature developmental time, higher immature mortality rate and the lower total fecundity, aphids had the lowest performance on the Fernor cultivar; the intrinsic rate of increase (r =0.0997 d-1), the net reproduction rate (R0 = 5.90 offspring) and the finite rate of increase (λ = 1.1048 d-1) values were the lowest, and mean generation time (T= 17.93 d) was the longest.

Keywords:*Panaphis juglandis, walnut, life table*

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Life table and predation analysis of predator-prey relationship between *Harmonia dimidiata* F. (Coleoptera: Coccinellidae) and *Aphis gossypii* Glover (Homoptera:Aphidiae)

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Life tables of the predator, Harmonia dimidiata F. and its prey, Aphis gossypii Glover, were studied using the age-stage, two-sex life table theory in order to consider the stage differentiation, variable developmental rate among individuals, and both sexes. To incorporate variation in predation rate due to the age, stage, and sex of predator, the daily number of A. gossypii preyed by individual H. dimidiata were also analyzed based on life table theory. At 25°C, the intrinsic rate of increase (r), the net reproductive rate (R0), and the mean generation time (T) for A. gossypii are 0.3953 d-1, 90.98 offspring, and 11.4 d, respectively. The values of r, R0, and T for H. dimidiata are 0.135 d-1, 283.02 offspring, and 41.6 d, respectively, when all eggs are included. Because the hatch rate of eggs of H. dimidiata varied with the age of the female, we also calculated the population parameters using only hatched eggs; the values of r, R0, and T for H. dimidiata are then 0.0904 d-1, 68.15 offspring, and 46.3 d, respectively. These results demonstrated the necessity of excluding the unhatched eggs to reveal realistic population parameters in cases where hatch rate varies with female age. The net predation rate of H. dimidiata fed on A. gossypii is 14,997 aphids. On average, 203.9 aphids are required for the production of a single fertile H. dimidiata egg. The computer simulation can be used for quantifying the number of predators necessary for successful control.

Keywords:*life table, predation rate, predator-prey relationship, Harmonia dimidiata, Aphis gossypii*

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Constructing life tables for difficult to rear species: A case study of *Forcipomyia taiwana* (Shiraki) (Diptera: Ceratopogonidae)

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Cohort life table begins with a batch of eggs. The developmental data from egg to adult emergence were collected first, and then the fecundity data were collected from females emerged from those eggs. For the biting midges, Forcipomyia taiwana (Shiraki) (Diptera: Ceratopogonidae), oviposition data could be recorded from field-collected female adults after blood meal in the laboratory, and the developmental data from egg to adult stage could be observed as well. It is, however, difficult to get the emerged females and males under artificial condition. Therefore, the cohort life table could not be constructed due to the difficulty of mating in laboratory. In this paper, I used random sampling to construct cohort life table by linking the oviposition data of parent generation and developmental data of the offspring generation. I validate this method by using a cohort life table of Aphis gossypii. With 30000 random sampling, the means of population parameters of constructed cohorts can take the variance of fecundity database into consideration. The reproduction database of F. taiwana was recorded from field collected females during Nov. 2009 to Apr. 2010 in Taichung, Taiwan. By using this method, the intrinsic rate of increase (r), the net reproductive rate (R0), and the mean generation time (T) for F. taiwana were 0.101 d-1, 14.72 offspring, and 26.63 d, respectively. This method can be used to construct life table for difficult to rear species with the advantage of estimation the variation of population at the same time.

Keywords:*Constructing life table, Forcipomyia taiwana, Aphis gossypii, reproductive database*

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The temperature-dependent development and life table parameters of *Aphis gossypii* Glover (Hemiptera., Aphididae) on *Gossypium hirsutum* L. using a linear and various nonlinear models

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Development rates, survival, fecundity and life table parameters of Aphis gossypii Glover (Homoptera: Aphididae) on Gossypium hirsutum L. at five different constant temperatures (15, 22, 25, 30 and 33 °C) and 60±5% RH were studies in the laboratory. The relationships between development rate of A. gossypii and temperature were described by fitting data to six nonlinear models and a linear model. Polynomial (P4), Logan 6, Logan 10, Janish, Briere 1 and Lactin 1 nonlinear models were used. The life tables of A. gossypii were constructed at the above-mentioned temperatures, except for 33 °C. For all stages, the relationship between temperature and development rate was predicted by the models. Euler-Lotka equation was used to estimate the age-specific life table parameters. The most appropriate curves of age-specific survival rates of adults (lx) was the Weibull frequency distribution. The estimated lower development threshold and optimal development temperature for A. gossypii were 8.8 and 30 °C, respectively. Cotton aphid required 92.4 degree-day to complete the development from first instar to adult stage. During the adult stage, the number of reproduced nymphs by a female generally reached the maximum in a few days and then slowly decreased to the end of oviposition period like most insects. This relationship was described by Enkegaard equation and most appropriate curves were fitted for fecundities (mx) at different temperatures.

Keywords:*Aphis gossypii, Gossypium hirsutum, temperature-dependent development, age-specific life table, Euler-Lotka equation, Weibull distribution, Enkegaard equation, Nonlinear Regression models*

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Life table of *Paederus fuscipes* (Coleoptera: Staphylinidae)

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The life table of Paederus fuscipes Curtis was studied under laboratory conditions using three field strains from Malaysia: Desa Wawasan (DW), Sri Pinang (SP), and Ampang Jajar (AJ). The total development time of pre-adult stage of DW was 17.43 ± 0.16 d, significantly different from SP (18.60 ± 0.19 d) and AJ (18.68 ± 0.22 d). Unlike SP and AJ strains, adults of DW have shorter life span. Due to shorter life span, the reproductive output of DW exhibited two peaks in age-stage specific fecundity, registering 121.28 ± 15.98 eggs compared to SP's (127.30 ± 18.01) and AJ's (147.45 ± 17.12). The intrinsic rates of increase (r) of P. fuscipes from DW, SP, and AJ were 0.0773 ± 0.0046 d?1, 0.0788 ± 0.0051 d?1, and 0.0873 ± 0.0054 d?1, respectively; and the net reproduction rates (R0) were 40.09 ± 7.39 offspring, 45.29 ± 8.74 offspring, and 42.34 ± 8.25 offspring, respectively. The mean generation time of P. fuscipes from AJ was 43.08 ± 1.07 d, which was significantly higher than that from DW (47.95 ± 1.36 d) and SP (48.57 ± 1.43 d).

Keywords:*rove beetle*

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Effect of host plants on life table of broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae)

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Development, longevity and reproduction of broad mite, Polyphagotarsonemus latus (Banks), on three host plants; white mulberry (Morus alba L.), basil (Ocimum basilicum Linn.), and physic nut (Jatropha curcas L.) were investigated under laboratory conditions (25.5-30.3 ℃, 44-81 %RH, 12:12 L:D photoperiod). Broad mite completed its development from egg to adult significantly slower on physic nut leaf disc (3.71 d) than on basil leaf disc (3.37 d), but not on mulberry (3.50 d). Broad mites reared on physic nut leaf disc (8.4 d) significantly lived shorter than those reared on other two host plants (mulberry, 10.9 d; basil, 11.1 d). After reached adulthood, female broad mites could stay alive alone on host plant leaf disc similar to male on the average in all host plant, and mostly produce only male (basil, 100%; physic nut, 79 %), with a few exception (mulberry, 43%). Without male, female lived 4.9 d, 7.7 d and 8.4 d and reproduced 4.5, 6.7 and 7.3 eggs per female on physic nut, white mulberry and basil, respectively. However, the intrinsic rate of increase (rm) of broad mite on basil was lower (0.0985 d-1) than on physic nut and mulberry (0.1472 d-1 and 0.2566 d-1, respectively). The net reproductive rate of broad mite were 2.39, 2.24, and 6.52 and mean generation time were 9.2, 5.4, and 8.1days, on basil, physic nut and mulberry, respectively. Adding male to female arena on physic nut resulted in higher rm (0.2221 d-1) and significantly produced more female (% female progeny, 59.2%).

Keywords:*Broad mite, life table, white mulberry, basil, physic nut*

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Population parameters of the oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) of different sex ratio treatments

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For a two-sex population, sex ratio plays an important role in population growth rate. In this study, I collected the life tables of the oriental fruit fly Bactrocera dorsalis (Hendel) with different sex ratio treatments. At sex ratio of 50♀:50♂, females began to lay eggs within 7 to 10 days after emergence, and mated 2.2 times during their lifespan, the net reproductive rate (R0) was 554.9 offspring, the intrinsic increase rate (r) was 0.1689 day-1, the average generation time (T) was 37.4 day. At a sex ratio of 50♀:1♂, males mated 53.3 ± 12.2 times during their lifespan, the females mated only once; both the adult pre-oviposition period and the total pre-oviposition period were longer than that of the sex ratio 50♀:50♂. At a sex ratio of 50♀:1♂, the R0 was 561.9 offspring, which is not significantly different from that of 50♀:50♂. But at 50♀:1♂, the intrinsic rate r was 0.1194 day -1, which is significantly lower than that of 50♀:50♂. The mean generation time (T) in 50♀:1♂ was 53.1 days; it was significantly longer than that of 50♀:50♂. Our results indicated that at a low male ratio, even though the B. dorsalis population had a lower intrinsic rate, the net reproductive rate was not affected. The effectiveness of a management strategy based on male annihilation needs to be further evaluated.

Keywords:*Oriental fruit fly, Sex ratio, Population parameters*

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Life tables of the tortoise beetle, *Aspidomorpha miliaris* (Coleoptera: Chrysomelidae) on Convolvulaceae

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The purpose of this study was to investigate the life cycle of the tortoise beetle, Aspidomorpha miliaris on five Convolvulaceae host-plants. The Convolvulaceae species chosen were water convolvulus (WC, Ipomoea aquatica), sweet potato (SP, Ipomoea batatas), mile a minute vine (MM, Ipomoea cairica), common morning glory (CM, Ipomoea purpurea), and littlebell (LB, Ipomoea triloba). The pre-adult stage of A. miliaris on WC, SP, MM, CM and LB lasted 41.40(±1.92), 40.63(±1.74), 41.98(±1.53), 41.03(±1.79), and 39.77(±0.81) days, respectively, at 250C. The adult longevity was 123.68(±26.63), 127.60(±19.54), 121.85(±12.86), 86.93(±9.81), and 111.08(±10.49) days, respectively, on these host-plants. The intrinsic rate of population increase (rm) was 0.04930, 0.05996, 0.05369, 0.04286 and 0.04810 and reproduction rate (R0) was 98.90, 145.87, 132.20, 50.57 and 75.60, respectively, on the five Ipomoea species. The mean generation time (T) was 93.19, 83.11, 90.97, 91.54 and 89.92 days and the finite rate of population increase (λ) was 1.0505, 1.0618, 1.0552, 1.0438 and 1.0493, respectively, on WC, SP, MM, CM and LB. All rm values were lower than 0.1. Among the host-plants, the cohort fed on sweet potato had the highest rm, R0 and λ; these values were the lowest when insects were fed on common morning glory.

Keywords:*population parameters, Aspidomorpha miliaris, Convolvulaceae*

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S1005M11

Integrated Pest Management

S1005

Use of life tables in aphid demography and in evaluation of crop resistance

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The life table is not only important in insect ecological studies, but also an efficacious tool in the evaluation of crop resistance to insects. We can use the life table to evaluate the effects of UV-B, heavy metals, high temperatures, and pesticides on aphids. We use also life tables to differentiate the biotype of aphids and to estimate the influence of treated plants on the aphids feeding. Our results showed that the intrinsic rate of increase r, mean generation time T, and net appreciation rate R0 are important indices. They can reflect the influences of environmental factors on aphids. Furthermore, 46 germplasm resources of wheat were identified by using the life table study. Aphid-resistant cultivars or lines of wheat were selected. According to the analysis of new XZ hybrids of wheat, the resistance mechanism was tolerant of aphids.

Keywords:*Life-Table, aphids, environmental factors, crop resistance*

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Life tables of important medical insects

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Life tables have been used to understand survival rate and fecundity of insect population. It is the most important basis for qualitative and quantitative study of population ecology. I reviewed the life tables of a number of important medical insects, belonging to five orders; Diptera (mosquitoes, sand flies, black flies, blow flies, flesh flies, horse flies, deer flies, stable flies, and house flies), Hemiptera (bed bugs and assassin bugs), Siphonaptera (fleas), Phthiraptera (sucking lice) and Dictyoptera (cockroaches). They are important not only to human and animal health, but also to the economy. Four categories of life tables were used: (1) "Lotka" female life tables, which collected age-specific survivorship and fecundity to compute population parameters; (2) field life tables, which collected survival and fecundity under field conditions; (3) statistical life tables, which borrowed from demography and the actuarial sciences to analyze the mortality experience of cohorts, and (4) age-stage, two-sex life-tables, which take both sexes and the variable developmental rate among individuals and between sexes into consideration. While using the first three methods, the followings errors or problems were frequently noted: (a) only females were included, (b) stage differentiation could not be described, (c) survival rate and fecundity were usually based on the adult age, (d) stage-specific control strategy was impossible. Based on this review, the age-stage, two-sex life-tables are the optimal way to analyze the life table data of medical insects.

Keywords:*Life tables medical insects*

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The effect of certain watermelon varieties on the biology of *Aphis gossypii* Glover (Hemiptera: Aphididae)

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The development, reproduction, and population growth parameters of the melon aphid, Aphis gossypii (Glover), on four watermelon varieties were evaluated in climatic room and growth chambers. The watermelon varieties were Crimson Sweet, Crimson Tide F1, Galactica, and Surme. Significant differences in the biology of Aphis gossypii were observed. The highest fecundity was recorded on Galactica (58.97 offspring/female), followed by Crimson Tide F1 (57.80 offspring/female), Surme (57.25 offspring/female) and Crimson Sweet (57.03 offspring/female). The highest net reproductive rate was observed on Crimson Tide F1 (22.40 offspring), while the lowest was on Surme (18.61 offspring).

Keywords:*Aphis gossypii, Biological parameters, Watermelon, Varieties*

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Consumption capacity of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) fed on asparagus determined by using the two-sex life table

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Consumption capacity of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) reared on asparagus (Asparagus officinalis L.) were studied under laboratory conditions by integrating into the age-stage, two-sex life table. Development, survival, fecundity, and consumption data were analyzed by the age-stage, two-sex life table. This study indicated that asparagus is a natural but suboptimal host of H. armigera due to the poor nutritional content in its foliage and poor fitness of H. armigera fed on it. The uncertainty associated with life table parameters were estimated by using jackknife and bootstrap techniques and the results were compared for statistical inference. The intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R0), and mean generation time (T) were estimated by the jackknife technique to be 0.0780 day-1, 1.0811 day-1, 67.4 offspring, and 54.8 day, respectively, while those estimated by the bootstrap technique were 0.0752 day-1, 1.0781 day-1, 68.0 offspring, and 55.3 days, respectively. The net consumption rate of H. armigera, as estimated by the jackknife and bootstrap technique, was 1183.02 and 1132.9mg per individual, respectively. The frequency distribution of sample means obtained by the jackknife technique failed the normality test, while the bootstrap results fit the normal distribution well. By contrast, the relationship between the mean fecundity and the net reproductive rate, as estimated by the bootstrap technique, was slightly inconsistent with the relationship found by mathematical proof. The application of the jackknife and bootstrap techniques in estimating population parameters requires further examination.

Keywords:*age-stage, two-sex, intrinsic rate of increase, net consumption rate, resampling*

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Effect of static magnetic fields (SMF) on the viability and fecundity of aphid *Sitobion avenae* (Homoptera : Aphididae) under laboratory conditions

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Using the age-stage two-sex life table, our experiment estimated the effect of two external static magnetic fields (0.176T and 0.065T) under four different exposure periods (15min, 30min, 60min, 120min), on the demography of Sitobion avenae in terms of development, fecundity and reproduction. It was demonstrated that nymphs exposed to these two magnetic fields statistically significant shorten the first instar and adult longevity, prolonged the third and fourth instar under the treatment for 0.176T-30min and 0.065T-60min in comparison with the control group. For the 0.176T-30min and 0.065T-60min, compared with the control (the first instar 4.0 d and adult longevity 8.4d), the results showed that the first instar was shortened by 1.0d and 0.9 d, adult longevity was shortened 3.9d and 3.1d; the total age of third and fourth instar were prolonged by 1.4d and 0.6d; but they were no interaction in exposure time and intensity of SMF. Lifetime fecundity of these two exposure rates were 4.6 and 0.9, or 23% and 5% of the control (19.9), but it was not showed statistical significance. The intrinsic rate of increase (r) were 0.1165 and -0.0198, net reproductive rate (R0) were 3.5 and 0.7, mean generation time (T) were 11.7d and 11.8d, and finite rate of increase (e) were 1.2382 and 0.9791 in comparison with the control group (r 0.2257, R0 19.8,T 13.2,and e 1.2531). These results can be attributed to the modulatory effect of the employed external magnetic field on development and growth regulation in the insects.

Keywords:*Static magnetic field (SMF), Sitobion avenae, life table, Homoptera, Aphididae*

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S1005M16

Integrated Pest Management

Notes on the biology of fig-tree moth *Ocnerogyia amanda* Staud. (Lepidoptera:Lymantriidae) from Erbil City, Kurdistan Region, Iraq

Wand Khalis Ali
University of Salahaddin, Iraq

This study was carried out in Erbil city for three years from 1-3-2009 to 19-11-2011 continually both in the field and laboratory belong to Directorate of Agriculture Research-Ankawa within Erbil city. The results showed seven generation per a year for the fig moth with eggs laid by a female ranged from 98 to 156 eggs, while the egg incubation period ranged from 7-12 days and the larval stage occupied 7-15 days and the pupal stage covers 7-11 days, more over the entire life cycle for all the studied generations ranged between 28 to 39 days. Also some field and laboratory observation were taken, in particular the length of duration of the separate larval instars, number of mating and number of generation per year, which seem not to have been given before in detail.

Keywords:*Fig-tree Moth Ocnerogyia amanda Staud., Insect Biology, Kurdistan Region*

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Effects of host range, mobility, and surrounding environment on spatial dynamics and movement of insect pests

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Spatial and temporal distribution of insect pest is one attribute that can assist in understanding its population dynamics and management. When the spatial and temporal pattern of insect pests is described, logical hypotheses about factors driving the distribution can be explored, which leads to science-based pest management. In this study, spatial dynamics of multiple insect pests with different host range, mobility, and surrounding environment were compared. A multi-year study was conducted to characterize the spatial patterns of the insect pests by using geostatistics and spatial analysis by distance indices (SADIE). For insects with narrow host range, field-scale study was conducted with an off-set sample lattice on 8-ha farms in Iowa, U.S.A. For insects with wide host range, samples were located in multiple crops on a 12-ha farm in West Virginia, U.S.A. Geo-coordinates of sample locations were obtained with a differentially-corrected global positioning system (DGPS; Magellan MobileMapper Pro, Santa Clara, CA). Spatial data obtained from this study were used to generate distribution maps of the insects in two ways: contour mapping with geostatistics and patch/gap mapping with SADIE. This study elucidated how insect pests move within and among commodities and how spatial dynamics of the insect pests were affected by mobility of the insect pests and availability of host plants. Different management options based on spatial dynamics, mobility, and host range of insect pests are discussed in this presentation.

Keywords:*Geostatistics, host range, spatial analysis, mapping*

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Entomological-radar monitoring of insect pest population movements

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The inland of south-eastern Australia encompasses extensive croplands and rangelands with no major barriers to aerial movement. This region hosts several migratory insect pests, including Australian plague locusts and several noctuid moth species. Populations arising in the rangelands following characteristically irregular rains migrate into cropping areas, causing damage that varies from year to year both in its intensity and in the region affected. Since 1999, insect migrations within this region have been observed with two fully-automated Insect Monitoring Radars (IMRs) located 300 km apart. These units operate throughout each night and detect individual insects as they pass directly overhead. A summary of the observations is available to locust forecasters the next morning, and a full data archive is maintained to allow more complete retrospective analyses. The IMR signals yield estimates of each target's height, speed and direction of movement, and alignment, along with measures of size and shape, and sometimes also the wing-beat frequency. The last few quantities are used to assign a target to one of a small number of classes, each representing a group of species - larger orthopterans, large moths, small moths, etc. Ancillary survey and trapping may identify the species predominating a class on particular nights. Significant movement events can be recognised for each class, and their intensities, directions, and spatial scales estimated. Statistics of these and other measures of migratory activity derivable from the radar data can be drawn on by pest forecasters and those making decisions about whether, when, and where to control.

Keywords:*radar, locust, moth, migration, Australia, forecast*

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Analysis of spatial distribution of greenhouse insect pests in Korea

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Analysis of Spatial distribution of two greenhouse insect pests, greenhouse whitefly and western flower thrips were evaluated using variogram models and spatial analysis of distance indices (SADIE) in Korea Variogram analysis was used to estimate and compare the attractive distances of two different sizes of yellow sticky traps for sampling whitefly adults in four commercial cherry tomato greenhouses. The patch size of whitefly immatures between plants was also estimated using visual counts. For whitefly adults on sticky traps the range of variogram was not considerably different between two trap sizes, which indicated that the attractive distances of the two different yellow sticky traps were very similar. The ranges of the variograms for the visual count of immatures on plants were always less than those for adults, indicating that the attractive distance of the traps for whitefly adults extends beyond the patch size for immatures on cherry tomato plants. The spatio-temporal dynamics of immature and adult of western flower thrips were analyzed based on the classical indices and SADIE. Based on the dispersion indices, thrips were found to be aggregated in both greenhouses. The SADIE index of aggregation and association probability were estimated and compared between stages and between sampling times. There were substantial spatial associations between adults and immatures each week; however, the temporal stability of the spatial patterns of adults and immatures decreased over time. The spatial patterns of thrips and their associations presented here provide the baseline information necessary to understand and predict plant damage and thrips dynamics.

Keywords:*Variogram models, spatial analysis of distance indices (SADIE), Patch sizes, Spatio-temporal Association*

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S1006M04

Integrated Pest Management

Influence of predator-avoidance behavior on the movement patterns of whiteflies in greenhouse

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This study focused on destructive crop pests, whiteflies (Hemiptera: Aleyrodidae), as a model system to explore how natural enemies can influence the efficacy of pest management programs. Towards this end, the study asked how predator-avoidance behaviors by whiteflies can improve and/or adversely impact the outcome of habitat manipulation and biological control. A series of manipulative experiments were conducted to test whether 1) predator-avoidance behaviors by whiteflies can be used as 'push component' to enhance trap cropping and 2) the predator-avoidance behaviors can unintentionally elevate pest dispersal from heavily-infested areas into uninfested habitats. First, we investigated under a trap crop system whether differential predation risks among plants influence host choice patterns of adult whiteflies, Bemisia argentifolii, between cash crop (poinsettia) and trap crop (cucumber). Among three natural enemy species tested, the predatory beetle, Delphastus catalinae (Coleoptera: Coccinellidae), on poinsettia induced significantly more whiteflies to abandon the cash crop and move onto the enemy-free trap crop. Second, we examined how adult whiteflies modulate resource uses in a response to the predatory beetles across different spatial scales. The results demonstrate that the magnitude of predator-avoidance behaviors by whiteflies could vary depending on available habitat scales. The presence of predators resulted in an elevated pest dispersal into new habitat areas within cucumber plant, whereas this predator effect was not measurable at among-plant movements by whiteflies. This study supports that non-consumptive effects of natural enemies should be further investigated to better understand these unexplored pathways between predator-prey interactions when designing and implementing bio-based management tactics.

Keywords:*Bemisia tabaci, Nonconsumptive effect, Dispersal*

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S1006M05

Integrated Pest Management

Site specific management of insect pests of elm trees in the University of Tabriz campus

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Elm trees are important landscape trees in most parts of the world including Iran. Like other plants many pests injure elms every year. Outbreaks of sucking insects weaken the trees and produce large amounts of honeydew contaminating clothes of passersby and cars parked under the trees. High volumes of broad spectrum insecticides are used to control these pests. The main objective of this study was site specific management of the elm trees using the University of Tabriz campus as a pilot. For this purpose, the campus was divided into 60×60 m grids and one tree was chosen randomly from each grid. Geographic coordinates of the selected tree was saved in a hand held GPS. The trees were sampled before spraying. Each sample consisted of a 30 cm branch terminal. At least eight samples were taken from each tree in four directions and two canopy levels. The number of leaves and all insects in the samples were recorded. The data were analyzed and the spatial distribution of the pests was determined. The regions with high populations were determined on the map. The trees with higher populations were sprayed with abamectin, imidacloprid and dimethoate and their lethal effects were compared. Each treatment consisted of four trees and three untreated trees were considered as control. The trees were sampled 1, 2, 4 and 7 days after treatment. Site-specific application reduced the insecticide input considerably. The results indicated that all insecticides reduced the pest populations significantly compared with control.

Keywords:*Elm trees, Site specific management, Insecticide*

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Spatial movements and dynamics of epidemic bark beetles

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The role of movements for spatial dynamics differs between the case of invasive bark beetles arriving in low numbers and bark beetles at epidemic densities. Two studies of bark beetles may illustrate some of the differences: (1) We use a generic model for the first step of the invasion process for bark beetles that may arrive in timber import to Scandinavia. Our results suggest that detection of dispersal from storage to forests will be difficult even when pheromone traps are used. This may imply that management actions are not to be deferred until after detection in nature, as the pest species is already established and eradication may be too late. However, pre-emptive measures reducing propagule pressure at one or several stages of the introduction process may strongly reduce introduction risk. (2) We model the sizes of outbreak patches by comparing model simulations based on dispersal of bark beetles and patterns of outbreak forest patches killed by Ips typographus in the High Tatra Mountains of Slovakia. Since the relative importance of attraction and site specific characters is unknown, we included three model variants to simulate dispersal and development of outbreak patches. For the highly mobile bark beetles, our results indicate that distance to previous outbreak patches and their sizes are only weak predictors of the sizes of new outbreak patches.

Keywords:*forest pest, bark beetle, spatial dynamics, detection, introduction, outbreak patch*

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A simulation model to predict the potential spread of the pine wood nematode, *Bursaphelenchus xylophilus* over a range of scales

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The pine wood nematode, Bursaphelenchus xylophylus, is the causal agent of the pine wilt disease. The species is native to North America where it is only a minor pest, mainly on non-native tree species. It has been introduced to several Asian countries (Japan, China, Taiwan, Korea) and to Europe (Portugal with some records in Spain). In most cases, the nematode has spread very rapidly despite control measures, and caused extensive damage to forests. If tree species are susceptible and climate is suitable, then the pine wood nematode can cause pine wilt and trees can die within a few weeks. Within forests and woodlands, the nematode is carried from one tree to another by adult beetles of the genus Monochamus whereas it can spread over long distances as a result of accidental transportation of infested materials. It is therefore important to understand the dispersal mechanism of the nematode and to determine the areas at risk. Here we present a spread model which combines short distance spread (to describe the dispersal of the vector) and long distance spread (to describe human transportation). This model was successfully used to predict the potential range expansion of the pine wood nematode and pine wilt disease in China. It also provides indications of which European ports are the most at risk for nematode introduction arising from international trade. The potential spread in Europe from the present invaded area in Portugal will be determined more accurately in the frame of the EU project REPHRAME (2011-2014).

Keywords:*Pine wood nematode, Bursaphelenchus xylophylus, Biological invasion, Monochamus, Spread model, Potential range expansion*

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Putting the brakes on the European corn borer cycle in the US: Bottom-up effect of genetically modified (GM) maize damps cycle amplitude

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In this presentation we discuss the spatial ecology of the European corn borer [Ostrinia nubilalis (Hubner) Lepidoptera: Crambidae] that once so devastated maize crops it cost the American economy an estimated $1 billion per annum. The population of O. nubilalis cycles as a result of a density dependent relationship with entomopathogenic parasite, Nosema pyrausta (Microsporida: Nosematidae). We consider whether landscape-level manipulations can be used to restrict the cycle amplitude of O. nubilalis beyond the limits of its natural regulator. We test for the bottom-up effect of genetically modified (GM) maize engineered to express Bt transgenes (i.e. Cry1Ab) that has been used across the Corn Belt since 1996. Time series from Minnesota (1963-2009) and Wisconsin (1964-2009) were analyzed using non-linear regression and wavelet analyses complemented with a spatial spline and mechanistic model to demonstrate the differences in population dynamics of O. nubilalis between the two states. The extent of regime change in the US Corn Belt where rates of transgenic Bt maize adoption varied were statistically quantified: the introduction of Bt maize explained cycle damping when the adoption of the crop was high (Minnesota) but oscillations were damped but continued to persist when Bt maize was used less intensely (Wisconsin). We conclude that host plant quality is key to understanding both epidemic persistence and the success of intervention strategies. In particular, the dichotomy in maize management between states is thought to limit the spatial autocorrelation of O. nubilalis to a correlation length of 275 km.

Keywords:*spatial ecology, agricultural entomology, Lepidoptera, maize, Bt,*

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Lepidopteran pest pressure in stone fruit tree orchards in Korea compared to other countries

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Apple, pear, peach and plum are the main stone fruit trees in Korea. Fruit boring lepidopteran pests are the major concerns for growers not only for the domestic market but also for exporting. Carposina sasakii, Grapholita molesta are the major fruit boring pests. Researches on pest population dynamics and managements are presented. Seasonal cycle and pattern of pest occurrence was not quite related to the tree phenology. However the annual population size of both pests was different among tree species. Recently, Grapholita dimorpha is increasing its awareness in various fruit orchards. From the published data and our recent sampling study, pest pressure of Oriental fruit moth, peach fruit moth and codling moth including G. dimorpha was compared relative to the management practices. Further, critical information needed for developing area-wide management concept was discussed, such as dispersal capability, insecticide resistance and habitat management for natural enemies. .

Keywords:*Grapholita dimorpha, pest pressure, codling moth, dispersal, resistance, natural enemy*

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Reduced pest status of lightbrown apple moth (*Epiphyas postvittana*) and native leafrollers (Lepidoptera: Tortricidae) on apple orchards in New Zealand

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Leafrollers are important pests of tree and vine fruit crops in New Zealand. The dominant species is the ubiquitous lightbrown apple moth (Epiphyas postvittana), followed by endemic Ctenopseustis spp. and Planotortrix spp. Up to the mid 1990s, leafrollers were one of the most important pests on apples, but their status has since changed to being of minor economic consequence in integrated fruit production (IFP) programmes. This change during the past 30 years was documented in four ways: pheromone trap catches of adult males, searches for larvae and pupae, quarantine pest inspection records of packed fruit, and examination of harvested fruit that had not received leafroller-active insecticides. Until the late 1990s, apples were grown under a calendar-based programme of broad spectrum pesticides, with up to nine applications per year. Leafrollers had resistance to organophosphate insecticides, there was minimal biological control and up to 47% of fruit was damaged in managed orchards. Several larval and pupal parasitoids were introduced in the 1980s, and an IFP programme was implemented in the late 1990s where organophosphates were replaced with selective products. Today, pheromone trap catches and larval finds of lightbrown apple moth are greatly reduced, although its wide host plant range means it is still relatively abundant. IFP orchards receiving 1-2 leafroller insecticides annually typically have <0.5% fruit damage at harvest, and even on trees with no insecticides, infestations are generally <3%. Leafrollers are currently managed to an extremely high degree by a combination of highly effective selective insecticides supplemented with improved biological control.

Keywords: *Tortricidae, Apple*

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The impact of invasive brown marmorated stink bug, *Halyomorpha halys* on IPM programs in eastern US fruit orchards

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Brown marmorated stink bug, BMSB, Halyomorpha halys (Stal) (Heteroptera: Pentatomidae) is an exotic insect pest introduced into North America around mid 1990's. Currently, the BMSB populations are reported from over 30 states and Canada. The biggest economic impact of this invasive insect is observed on various agricultural and horticultural crops grown in the mid-Atlantic region of US. During last few seasons, some stone and pome fruit orchards experienced up to 60 percent loss of fruit value due to the BMSB feeding. At this time, in the absence of specialized effective natural control agents, only the use of broad spectrum insecticides provides adequate control of BMSB infestation. However, additional applications of insecticides, not normally used (or recommended) in our fruit IPM system, already are causing an increase in the number of observed outbreaks of secondary fruit pests such as mites, wooly apple aphids or scale insects. There is immediate and urgent need to develop and evaluate other, alternative methods and products that are effective against BMSB so softer, more sustainable methods can be utilized in the future. In our presentation we will attempt to present the established pre-BMSB fruit IPM system in Pennsylvania orchards and the actual and potential consequences of this accidental introduction of a single invasive pest species on common fruit IPM practices.

Keywords:*brown marmorated stink bug, Fruit IPM*

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Assessment of mass trapping with kairomones and pheromones against the apple clearwing moth, *Synanthedon myopaeformis*

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Semiochemical-baited mass trapping was tested against the apple clearwing moth (Synanthedon myopaeformis (Borkhausen) using pheromone-and kairomone-baited traps. Mass trapping significantly reduced the number of moths captured in assessment traps positioned in pheromone-treated but not kairomone-treated plots. Pheromone and kairomone-based mass traps removed more moths with increasing trap density. Kairomone mass trapping removed male and female moths and removed as many or more individuals from the population than pheromone-based mass trapping. Traps targeting the apple clearwing moth also captured non-target arthropods. More non-target arthropods were captured in kairomone-baited traps than pheromone traps. More non-target arthropods and a greater diversity of non-target arthropods were captured in organic than conventional orchards. Potential effects on non-target arthropods should be considered in future development of semiochemical-based management of the apple clearwing moth.

Keywords:*pheromone, kairomone, mass trapping, Synanthedon, apple*

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Considerations in the design of mass trapping or lure and kill for pest management

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Mass trapping and lure and kill are related tactics for male removal using female sex pheromones or other semiochemicals, and can offer pest management benefits. Mass trapping enables researchers to directly count the insects removed but the cost of traps and labour can be limiting. Lure and kill offers a solution to this but it can prove difficult to assess population suppression, due to competition between traps and wild female insects. Insights gained from mass trapping can indicate whether potential exists for lure and kill systems to be developed. A theoretical framework has been developed and tested on four different orders of pest insects (Lepidoptera, Homoptera, Diptera and Thysanoptera), in order to determine whether a standard protocol could be used to advance the development of mass trapping and lure and kill systems. The system is based on an assessment of trap competition and takes into account trap spacing, lure strength and other factors.

Keywords:*pest management*

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Biologically based management of Asian citrus psyllid (ACP) *Diaphorina citri* to reduce spread of huanglongbing in Florida

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Diaphorina citri Kuwayama vectors Candidatus Liberibacter asiaticus, a bacterium which causes Asian form of "huanglongbing" (HLB) or citrus greening disease. ACP and HLB discovered from Florida in 1998 and 2005, respectively, are now well established and pose a serious threat to the citrus industry. Vector control is critical to reducing the incidence of HLB. Biological control is critical to manage this pest disease complex. Field studies in 2006-2007 showed 5-27 fold reduction in psyllid populations mainly by predacious insects and spiders. Mature citrus trees in Florida produce most new growth in spring, followed by summer and fall which is attractive to pests and beneficial insects. Therefore, foliar sprays of broad spectrum insecticides applied to mature trees in winter were evaluated in commercial citrus as tactic to reduce psyllid populations and insecticide use in spring and summer. Single sprays of chlorpyrifos, fenpropathrin and oxamyl in January reduced adult psyllids an average of 10-15-fold over 5-6 months compared to untreated trees, respectively, without additional sprays. Spiders, lacewings, and ladybeetles were equally abundant during the growing season in both treated and untreated trees. This tactic has been adopted area-wide to manage psyllid in Florida. For additional suppression of psyllid we are mass producing and releasing Tamarixia radiata, an ectoparasitoid of ACP that we imported from Pakistan, South China, and North Vietnam in 2008 and already established strain imported from Taiwan and South Vietnam in 1999. The repeated releases of these parasitoids in citrus groves are contributing to overall reduction in psyllid population.

Keywords:*Diaphorina citri, huanglongbing, dormant sprays, broad spectrum insecticides, Tamarixia radiata*

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New emerging insect pests and their management strategies in citrus orchards in Jeju, Korea

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The arthropod pest status in citrus orchards has been largely changed since last decades in Jeju, Korea; probably caused by climate changing. We will introduce two new insect citrus pests of Epuraea domina Reitter (Coleoptera: Nitidulidae) and Scirtothrips dorsalis Hood (Thysanoptera: Thripidae) in this presentation. E. domina has been known as a pollinator in citrus orchards. During pollination activity, however, the appendages such as legs cause a scratch on the ovary of citrus flower, and it develops later to a corky scar on citrus fruits at harvest resulting in deterioration in the fruit quality. This insect has a very characteristic life cycle, showing winter breeding behavior. E. domina adults spend summer season in mountainous habits and migrate to citrus orchards in late autumn to lay eggs in soil during the winter. The hatched larvae feed on fallen citrus fruits during the winter to develop pupae. The pupae emerge to new adults in mid to late May to feed pollens and nectar in citrus blooming season; followed by migration again to mountainous habitats in early summer. Because they reproduce during the winter, weather condition in winter was essential for the population increase. S. dorsalis induces silver-grey, dark brown scaring or dark brown discolored damages on Mandarin citrus fruits in Korea, which has occurred in Jeju Island since 2007. Also, this pest has been known as causing economic loss on cash crops worldwide including Unite States. The population dynamics will be presented in relation to survival on host plants and weather conditions.

Keywords:*Epuraea domina, Citrus, Life cycle, Seasonal migration, Scirtothrips dorsalis*

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Progress in the development of an in-canopy fixed spraying system for high-density apple orchards

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Introduction: Pesticide application to tree fruits using airblast sprayers can be inefficient and inaccurate, resulting in spray drift, off-target contamination, and ineffective pest control. A spray system fixed into the tree canopy was used to apply pesticide sprays to a commercial planting and compared with a conventional sprayer. Methods: A high-density apple planting (0.5 ha) received all its pesticide sprays through a system of microsprinkler nozzles attached to polyethylene tubing running along the tops of tree canopies and supplied by a central tank and pumping station. In initial trials, insect and disease control, and spray deposition data were compared with those in a section of orchard treated using a tractor-pulled airblast sprayer. An improved design uses tubing reservoirs at each spray head, which are first filled with sufficient spray solution to adequately wet the surfaces of the foliage and fruits below the nozzles, and compressed air is then used to completely blow out the spray. Results: In the initial trials, sprays were applied quickly and efficiently, each taking only a few minutes to completely cover all the trees in the fixed-spray plots. In-season sampling sessions revealed comparable control levels of insects and diseases in both spray treatments. Continuing work will focus on optimizing efficiency of spray coverage and distribution using practical and economical system design considerations to encourage adoption by the fruit producing industry. Conclusions: A fixed nozzle arrangement could offer benefits in pesticide application efficiency and accuracy while maintaining crop protection efficacy and production quality in modern apple plantings.

Keywords:*pesticide application, pest control*

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IPM on top fruit in Italy: State of the art and outlook

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IPM was born in Italy in 1972, after ten years of entomological researches and investigations aimed to study the behaviour of the most important species of pest and beneficial insect and to assess the response to different control techniques. In those years, many demonstration projects began and achieved significant results and a substantial decrease in number and toxicity of the insecticides used. In the eighties, those projects turned into programs for Integrated Pest Management (IPM), and over time other fruit and horticultural crops joined to the programs. These crops are currently regulated by Integrated Production Disciplinary (DPI), which defines the crop growing techniques in all agronomic aspects and control strategy (sensu lato). The environmental factors were decisive in implementing IPM, especially since the early nineties, following specific Community Directives, which over time have allowed to increase IPM techniques until they become mandatory in Europe in 2014 (Law 128/09). The Directive emphasize the training and transfer of all the knowledge related to the proper use of insecticides, the general principles of IPM and the risk indicators that allow to assess the way in which chemicals are used. The market demands for productions without or with a limited number of residues might become a factor conditioning the IPM, stimulating, however, the improvement.

Keywords:*IPM, Italy, top fruits*

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S1007TU10

Integrated Pest Management

A 20 year evolution of pear pest management in the Western United States

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The history of the evolution of pear pest management in the Western United States from sole reliance on broad-spectrum organophosphate and pyrethroid insecticides to a codling moth pheromone mating disruption based system with supplemental reduced risk insecticides will be discussed in relation to causal factors responsible for this dramatic shift. The impact of this transition on key secondary pests and on resurgence of dormant pests will be discussed along with future directions of the Western United States pear pest management.

Keywords:*Pear Pest Managment*

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S1008TU01

Integrated Pest Management

Ecological control of native insect pests based on insect population genetics in regional rice IPM

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Yellow stem borer (YSB), Scirpophaga incertulas Walker, and striped stem borer (SSB), Chilo suppressalis Walker, are prevalent and destructive natives generally concurring in extensive rice-planted area along the Yangtse River of China. But in some patches of the area the either or both have occurred little for years. What acts as naturally sustained counteraction on the pests there? Our investigation found that, 1) breadth of yearly temporal niche in each of 11 YSB populations was greatly jumpy during past decades (for example, 0~0.68 or 0.43±0.19 in Suzhou of Jiangsu province for the last 34 years) corresponding to fluctuation of the population size in different years and significantly lower than that in the same site SSB (0.60~0.87 or 0.73±0.09), whereas breadth of the spatial niche is relatively much stable and high in that there existed little resistance of rice varieties and low parasitic rate (27.8% ~40.3%) of the Trichogramma japonicum Ashmead to YSB. Evidently the temporal niche of YSB is susceptible (but the spatial resistant) to alteration of the coevolutionary factors,while that of SSB quite stable. 2) The SSB stable niche derived partly from distinct temporal ecotypes including earliest (E) and later (L) types, to sampled individuals of which all showed specific DNA SCAR or AFLP markers (L820 to the L-type and E335 to the E). 3) The YSB susceptible niche broadened only providing rice duration in field extended longer than YSB yearly occurring duration or the latter was elastically compressible in certain zones or years with an incomplete generation of YSB.

Keywords:*rice stem borer, susceptible ecological niche, ecotype, population genetical management*

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S1008TU02

Integrated Pest Management

Ecological regulatory mechanism of temperature on population size in *Laodelphax striatellus*

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Temperature is one of the key factors that influence the occurrence of Laodelphax striatellus (Fallen), especially in the winter and summer. However, the specific biological responses of L. striatellus to high temperature were unclear. The effects of duration of high temperature (33 ºC) on life-history traits and morphological variation were studied using 27 ºC as the control. Exposure of adults for one day during oviposition stage led to a very low survival of nymphs. The average longevity of L. striatellus exposed for 1-31 days from oviposition was significantly longer than that of the control. Short-term (1-5 days) heat exposure from the 3rd instar nymphs did not significantly influence eclosion, but exposure from the 4th instar nymphs significantly increased eclosion. Lifespan from egg to adult was significantly lengthened when the 3rd instar nymphs were exposed to heat for 2~15 days, or the 4th instar exposed for 10 days. The preoviposition period were prolonged by the heat exposure from the 3rd or 4th instar nymphs. Short-term heat exposure of less than three days from the 3rd or 4th instar nymphs did not restrict fecundity, but when the exposure duration exceeded five days the total eggs per female and hatchability decreased. Exposure to high temperature increased the brachypter rate of adults and decreased fluctuating asymmetry in several nymphal morphologic traits. Low survival and slowing development under heat exposure resulted in population decline in the summer, and the relatively high fecundity, brachypter rate and symmetry led to quick revival in the autumn.

Keywords:*Laodelphax striatellus, heat exposure, Development, fecundity, wing form, fluctuating asymmetry*

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S1008TU03

Integrated Pest Management

Biological and ecological control of the brown planthopper in South China

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A community's capacity for development resides in its species pool. Species pool in rice ecosystem includes ridges of rice fields, roadsides, non-rice crop habitats and fields with no crops. Our results showed that the dominant species of natural enemies in ridges were often the same as those during reestablishment period of arthropod community in rice fields. In Guangdong Province, the spider Ummeliata insecticeps first moved from species pool into rice fields in early rice season and became the dominant species of the arthropod community. It had the biggest time-space niche overlapping value with rice planthoppers (including the brown planthopper Nilaparvata lugens), followed by Pirata subpiraticus. As one of the model examples, the rice IPM program in Dasha Township of Guangdong Province, located in N23°19', E112°40', started in 1973 in 1.6 ha rice field and reached 4000 ha in 1975. This long-term program has been very successful and achieved significant long-term benefits. In order to ensure the program successful, IPM experts teach farmers basic IPM knowledge such as distinguishing insect pests and natural enemies in a field farmer school (FFS); several biological and ecological control measures have been used for more than 30 years.

Keywords:*biocontrol, ecological control, brown planthopper, Nilaparvata lugens*

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S1008TU04

Integrated Pest Management

Diversity analysis of symbiotic bacteria in *Nilaparvata lugens* with different virulence

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Symbiotic bacteria are key players in insect-plant interactions influencing many aspects of insect ecology and playing a key role in shaping the diversification of many insect groups. The brown planthopper (Nilaparvata lugens Stal, Homoptera: Delphacidae) is an important pest of rice, which feeds mainly on the rice stems and sucks assimilates from the phloem. Different virulent population of brown planthopper has proven different virulent to the resistant rice varieties. Analysis of the diversity of symbiotic bacteria in Nilaparvata lugens with different virulence will be helpful for understanding the mechanism of virulence differentiation of brownn planthopper. Investigations of the diversity of bacterial communities associated with three virulent labortory populations(TN1,Mudgo and ASD7) and Zhejiang field population of the BPH were made using 16S rDNA-RFLP. Sequence similarity of 97% was used as the definition of operational taxonomic unit (OTU). We found the BPH harbours a variety of symbionts which belonged to 23 OTU including intracellular symbiotic Arsenophonus, Wolbachia and extracellular symbiotic Acinetobacter, Serratia sp., Arthrobacter sp., Chryseobacterium, and other uncultured bacterium. The densities of different symbiotics in different virulent population were analysed by real-time quantitative PCR with genus-specific primer. The results showed that the amount of Arsenophonus in TN1 population was higher than in other populations. The rate of Arsenophonus infection was 53.0% and 2.0% in TN1 and Mudgo population, respectively. Compare to the subpopulation

Keywords:*Nilaparvata lugens, Symbiotic,Virulence*

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S1008TU05

Integrated Pest Management

Recent advances in the management of insect pests of rice in the USA

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The two most important insect pests of rice in the U.S. are the rice water weevil, Lissorhoptrus oryzophilus, and the rice stink bug, Oebalus pugnax. There have been a number of important recent advances in the management programs for these two insects. An overview of these recent advances will be presented. Topics will include the use of seed treatment insecticides, development of thresholds, and the integration of constitutive and inducible plant resistance into management programs.

Keywords:*rice, integrated pest management, rice water weevil, rice stink bug, seed treatments, thresholds, plant resistance, induced resistance*

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Use of natural enemies as a bio-indicator for assessing environmental soundness of agricultural practices in rice paddies

Takatoshi Ueno
Kyushu University, Japan

Rice production in paddy fields can depend on pest control by natural enemies, in particular, when environmentally sound farming is applied. Maintaining the diversity and abundance of beneficial natural enemies should therefore be key to improving the productivity and sustainability in rice paddies with environmentally sound practices, and may also improve their function as an important habitat for endemic species living in wetland environments, many of which are now endangered or threatened. Use of natural enemies as indicator species to monitor and evaluate environmental soundness of farming practices will be a useful tool to promote sustainable rice production and conserve other valuable species, if the abundance and diversity of natural enemies can reflect farming practices and general biodiversity in rice paddies. Here I focus on arthropod natural enemies in rice paddies and examine the relationships among selected natural enemy species, farming practices (insecticide & manure applications) and general biodiversity. The diversity of parasitoids and predators reflected farming practices (e.g., pesticide and mature use) and the diversity of neutral species. Some of natural enemy species were found useful to assess general diversity and farming practices. The results have demonstrated that several species of parasitoid wasps and spiders are good biological indicators to monitor and assess biodiversity and environmental soundness of practices in rice paddies. Such bio-indicators can be used just like a 'bathroom scale' to know the healthiness of rice paddies.

Keywords:*biodiversity, parasitoids, predators, environmentally sound agriculture, organic farming*

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S1008TU07

Integrated Pest Management

Resistance management for planthoppers and leafhoppers in South East Asia: Challenges and opportunities

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Host-plant resistance has been the main focus for sustainable hopper management in rice for the last several decades. Currently over 70 resistance loci have been identified in rice and many have associated DNA markers that can be used in marker-assisted breeding programs. However, hoppers are known to quickly adapt to resistant varieties and resistance genes. This talk reviews the knowledge gained from over 30 years of research into hopper adaptation and highlights the challenges and opportunities for developing resistant varieties and reducing the incidence of hopper damage to rice. Patterns in adaptation differ considerably between planthoppers and leafhoppers and, though adaptation is often quicker in leafhoppers compared to planthoppers, results suggest that resistant varieties may be more durable against the former. It is clear that increasing hopper virulence is rapidly depleting the utility of available resistance loci in rice and that a coordinated attempt to manage virulence at a regional scale will be required to guarantee the success of future breeding programs.

Keywords:*Host-plant resistance, Virulence*

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S1008TU08

Integrated Pest Management

Enhancing biological control of rice planthopper pests by ecological engineering in China

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The effects of planting sesame on the bunds of rice fields on biological control of rice planthoppers was conducted in Jinhua, China. A 35ha experimental site was divided into two parts, in which 8 ha was established as an ecological engineering area with a total of 600 m2 of sesame planted on bunds, preserving biodiversity in non-rice habitats and reducing pesticide use. A 27 ha area was managed according to normal farm practice with conventional insecticidal control. Numbers of Mymaridae on yellow sticky traps in eco-engineering fields were at least four greater times than that in farmers' fields. Differences were especially great, up to 100 fold higher, in samples taken after pesticide application in the farmers' fields. The number of invertebrate predators including odonata was significantly higher in the eco-engineering area than the farmers' fields. Frogs too were much more abundant in the eco-engineering area. In contrast to the absence of use of pesticides in the eco-engineering areas, the conventionally managed comparison fields required spraying in mid-September to control escalating numbers of planthoppers. The number of planthoppers in the eco-engineering fields remained low. Results show that planting sesame on the bunds of rice fields to provide floral resources, combined with preserving areas of non-crop vegetation and withholding insecticides can increase natural enemy numbers and activity, translating to effective biological control of rice planthoppers. This work constituted one replicate block in a much larger experiment that extends across several countries and in which similarly positive results are being

Keywords:*rice pests, bunds of rice field, planting sesame, parasitic wasps, natural control*

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Horizontal webs of *Tetragnatha* spiders enhance biological control of insect pests by spiders in organic paddy fields

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Our observational study indicated that Tetragnatha spp. spiders, abundant in organic paddies, suppress a mird bug density and its damage level (Takada et al. 2012). However, the molecular gut content analysis showed that only a small proportion of the spiders fed on the bugs (Kobayashi et al. 2011). In order to understand how the cascading effect by Tetragnatha spp. spiders is enhanced, the following hypothesis was examined. The mirid bug Stenotus rubrovittatus, a major rice pest in Japan, usually remains in the upper layer of paddies to feed on rice ears. However, the mirids are frequently observed falling to the bottom layer of paddies after becoming tentatively trapped by horizontal webs of Tetragnatha spp. spiders. Bugs falling to the bottom layer are likely to be predated by ground-dwelling predators. It is hypothesized that Tetragnatha spp. spiders facilitate bug predation by wolf spiders through trait-mediated effects, in which their horizontal webs force the bugs onto the ground and thereby into the hunting zones of wolf spiders. Molecular gut content analysis of 619 wolf spiders revealed that the number of wolf spider individuals that tested positive for mirid bug predation increased significantly with Tetragnatha spp. density in the paddies. We also observed a positive relationship between Tetragnatha spp. density and total cover by their webs in paddies. We identified the potential for an unexpected interaction between an insect pest and ground-dwelling spiders that usually inhabit different microhabitats in paddy fields by focusing on trait-mediated effects of webs built by Tetragnatha spp. spiders.

Keywords:*ecosystem function, generalist predator, molecular gut content analysis, trait-mediated effect*

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S1008TU10

Integrated Pest Management

Genetics and breeding of rice conferring resistance to planthoppers and leafhoppers

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Insect pests are an important constraint in rice production in Asia. Among them, brown planthopper (BPH), Nilaparvata lugenes and green rice leafhopper (GRH), Nephotettix cincticeps are the most serious insect pests of rice in tropical and temperate Asia, respectively. Host-plant resistance is considered to be an economically, ecologically acceptable and practical approach for hoppers management. Several major resistance genes and quantitative trait loci associated with hoppers resistance have been identified in cultivated and wild relatives. Some of them have been transferred to desired genetic backgrounds to develop resistant varieties. Here we present some examples of identification of BPH and GRH resistance genes (i.e., BPH3, BPH4, BPH25, BPH26, GRH1, GRH2, GRH4, GRH5, and GRH6) and development of rice near-isogenic and pyramided lines with the resistance via marker-assisted selection. Through this approach, the resistance genes have been transferred to new genetic backgrounds and several unfavorable alleles linked to the target genes have been removed. The near-isogenic lines carrying the resistance genes should be useful to monitor the virulence of rice hoppers. The pyramided lines were significantly resistant to hoppers. This gene pyramiding strategy should be useful in rice breeding to develop broad-spectrum and durable resistant varieties.

Keywords:*rice, brown planthopper, green rice leafhopper, marker-assisted selection, resistance gene*

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S1008TU11

Integrated Pest Management

Virulence monitoring and integrated management for main rice insect pests in South China

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This paper outlines recent research advances in virulence monitoring and integrated management for main rice insect pests in south China. Three components were discussed: (1) Monitoring the variation of the Virulences of main rice insect pests including brown planthopper Nilaparvata lugens (stal), whitebacked planthopper Sogatella furcifera (Horvath) and rice gall midge Orseolia oryzae Wood-Mason. (2) Evaluation and application of rice resistant germplasms and varieties to brown planthopper, whitebacked planthopper, rice gall midge. (3) Technologies and strategies of IPM for main rice insect pests in south China.Three key techniques were implemented: (a) resistant varieties, (b)cultural control and (c) judicious use of insecticides and protection of helpful insects and spiders.

Keywords:*brown planthopper, whitebacked planthopper, rice gall midge, virulence, resistant varieties*

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S1008TU12

Integrated Pest Management

Silicon-mediated rice resistance to the striped stem borer: Effects of rice varieties and larval stage

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The rice stem borer Chilo suppressalis is one of the most destructive pests in rice. The aim of this study was to investigate the potential of silicon addition in mediating rice plant resistance to first and third larval instars of C. suppressalis on a susceptible (Shanyou63) and a moderate resistant (Yanfeng47) rice variety. Potted rice plants were treated with calcium silicate at rates of 150 kg/ha (SiO2) or 600 kg/ha (SiO2) or left untreated (the control). Si amendment significantly increased Si content in rice stems, rice variety and interaction between Si treatment and rice variety also significantly influenced SiO2 content in rice stem. Percentage of larvae bored (PLB) decreased 5% or 28% with increases in Si addition. Percentage of larvae penetrated (PLP) did not differ among Si treatments or between rice varieties for the first instars; for the third instars, PLP significantly decreased 10% or 40% with increases in Si supply and was 10% to 30% lower on Yanfeng47 than on Shanyou63. Si addition significantly prolonged penetration duration (PD) in the first instars and in the third instars, PD was longer on Si+ Yanfeng47 than on Si+ Shanyou63. Analysis showed that increased SiO2 content in rice stems due to Si addition contributed to the reduction in PLP and prolong in PD of the third instar larvae. Silicon treatment significantly decreased larval development, weight gain and stem damage. Some of the effects were manifested more strongly in the susceptible rice cultivar compared with the moderate resistant cultivar.

Keywords:*silicon, Chilo suppressalis, rice, plant resistance, boring behavior, performance*

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Possible invasion areas of the alien apple snail, *Pomacea canaliculata*, estimated from accumulated climate data by automated meteorological data acquisition system (AMeDAS) in Japan

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The apple snail, Pomacea canaliculata, has become a major pest of rice in Japan, representing an important constraint on the implementation of direct seeding. Anticipating possible invasion areas is expected to prevent further spread. Cold weather is a major factor restricting its distribution in Japan. Mortalities increased depending on low temperatures below 10oC and exposure time. Therefore, we used cumulative low temperatures below 10oC during winter (CLT10) as an index to determine whether or not the snails can overwinter. Snail distributions were surveyed in several regions and CLT10 at each location calculated. The climate data at the specific locations was estimated from AMeDAS, using the software (Mesh2000). As a result the maximal CLT10 in which the snails can overwinter was estimated to be 700 day-degrees. We drew the areas below 700 day-degrees on a map as the possible invasion areas. The areas extended both regions of west Japan facing the Pacific Ocean and the Japan Sea. However, at present we found no snail distribution in the areas facing the Japan Sea except one location where hot-spring comes out. Furthermore, for the last 20 years snails in this place did not invade surrounding areas blow 700 day-degrees. Therefore we considered snow fall, another factor besides CLT10, which prevents overwintering, because temperatures are kept long at 0oC under snow. Thus, the areas where CLT10 are below 700 day-degrees together with the maximal snow fall being below 20 cm were drawn as the tentative map of possible areas invaded by P. canaliculata.

Keywords:*Pomacea canaliculata, Golden apple snail, Invasion, Overwintering, Climate, AMeDAS*

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S1009TU01

Integrated Pest Management

Looking back to move forward: Historical milestones of host plant resistance in integrated pest management succession

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Host plant resistance has long been part of crop protection with the first report of Hessian fly-resistant wheat cultivars in 1782, six years after the first report of a pest on wheat in the USA. The use of chemical insecticides proliferated worldwide during the 1940's through the 1960's because they were inexpensive and so effective that all other insect pest management tactics on crop plants, including host plant resistance, received little support. The overuse of insecticides resulted in outbreaks of insecticide-resistant insect pests, emergence of secondary pests to pest status, and the bioaccumulation of chlorinated hydrocarbon insecticides in the environment. This led to the re-examination of alternative tactics to chemical control and the concept of integrated pest management (IPM) as control strategy. In addition to host plant resistance to insects and diseases, substantial progress has been made using biological control tactics such as parasitoids, predators, and entomopathogens to manage insect pests. The introduction of transgenic crops (e.g., Bt-transgenic corn and cotton, etc.) has profoundly changed the concept of IPM. Interlinking the principles of genetics and ecology will profoundly impact our ability to protect crop yield and quality in the transgenic era.

Keywords:*host plant resistance, ecological genetics, cisgenic and transgenic crops, IPM*

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Holistic corn breeding approach to improve abiotic and biotic and stress tolerance

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Corn earworm, Helicoverpa zea (Boddie), is one of the major economic pests of corn both in the U.S. and worldwide. Its larvae feed on the corn silks and kernels, which increases grain mold by such fungi as Aspergillus flavus and enhances aflatoxin contamination in grain. Drought and heat stress reduces yield and increases aflatoxins. Our strategy to improve abiotic and biotic stress tolerance is to explore desirable native genes from exotic germplasm and wild species and develop multiple stress tolerance corn germplasm. The tropical x temperate breeding crosses from the Germplasm Enhancement of Maize (GEM) project are a useful source for selecting abitotic and biotic stress tolerant corn. Inbred CML343 and its hybrids are highly resistant to corn earworm. We have developed inbred lines from crosses between temperate x tropical corn or between corn x teosinte. Results showed that several experimental hybrids had high yield and significantly lower aflatoxins as compared to commercial hybrids. Improving host plant resistance to corn earworm and drought tolerance can be an effective approach to the risk of aflatoxin accumulation.

Keywords:*Host plant resistance, maize, breeding, abiotic and biotic stress tolerance*

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S1009TU03

Integrated Pest Management

Genetic basis of wheat resistance to the English grain aphid

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The English grain aphid is an important pest in wheat production worldwide. Development of aphid-resistant wheat and cereal varieties has been considered as one of the effective aphid management strategies in wheat and cereal production, which could reduce the use of synthetic insecticides, and improve crop yield and quality. A series of experiments were conducted to determine aphid resistance machenismms and understand the genetic basis for aphid resistance. After massive screening, 23 varieties were seleted from 2340 wheat germplasm as resistant to S. avenae. In particular, the varieties in XZ series, '98-10 ? 30 and '98-10 ? 35' were the best in aphid resistance among the selected wheat germplasm lines. Four linked SSR markers Xgwm350, Xbarc70, Xwmc702 and XCfd46 were identified, which were then used for aphid resistant gene mapping on 7DL chromosome in wheat. The distance of the four markers were 6.3 cM, 15.1 cM, 19.1 cM and 34.1cM on chromosome 7DL. Aphid resistant gene in wheat germplasm '98-10 ? 35' was determined on chromosome 7DL, which was designated as Sa1. Furthermore, aphid resistance in the F3 of 98-10-35/1376 was analyzed using DDRT-PCR technique. The banding patterns related to S. avenae resistance in the F3 plants could be categorized as the following five categories: 1) bands occurring in only parents; 2) bands occurring in only one parent; 3) bands visualized in only hybrid; 3) bands expressed in both hybrid and the resistant parent; 4) bands in the offspring being up-regulated; and 5) bands in the offspring being down-regulated.

Keywords:*Wheat, Aphid resistance, Resistance gene*

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Specific resistance of eight wheat varieties to *Sitobion avenae*, *Rhopalosiphum padi*, and *Schizaphis graminum* (Hemiptera: Aphididae)

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Sitobion avenae, Rhopalosiphum padi and Schizaphis graminum were the three most destructive wheat pests worldwide. Many commonly used wheat varieties exhibited different resistance to different aphid species. We evaluated the resistance of eight varieties based on their negative (resistant), positive (susceptible), or no effects to the three aphid species using six parameters, development days, fecundity, weight difference between 1st instar and newly emerged adult, offspring per female produced per day, relative growth rates, and nymphal survival. We found that the effects of the eight varieties to the three aphid species differed significantly. The varieties, WW2730 and Astron were resistant to Sitobion avenae and R. padi, but susceptible to Schizaphis graminum; Batis was resistant to Schizaphis graminum, but susceptible to Sitobion avenae and R. padi; Amigo was only resistant to Schizaphis graminum; 98-10-30 was resistant to Sitobion avenae; Xiaoyan22 was resistant to R. padi, and had negative effects on the reproduction of Sitobion avenae and Schizaphis graminum, but had positive effects on the growth of Schizaphis graminum; 186tm was susceptible to all three aphid species, but caused the highest mortally to Schizaphis graminum and Sitobion avenae. The variety, 98-10-35 had negative effects on the reproduction of Sitobion avenae and development of R. padi, but caused the highest mortality to R. padi. We concluded that the wheat varieties have specific resistance to different aphid species. And the "trade-off" of host resistances exists on the wheat varieties level. Our data should be useful for aphid management and wheat breeding for aphid resistance.

Keywords:*Sitobion avenae (Fab), Rhopalosiphum padi L. , Schizaphis graminum (Rondani), Wheat varieties, Resista*

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Native host plant resistance to whiteflies in watermelon

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Whiteflies (Hemiptera: Aleyrodidae), notably Bemisia tabaci (Gennadius), are destructive pests on a worldwide scale. B. tabaci feeds on over 1,000 host plant species with varying levels of infestations. This pest is a problem in cultivated watermelon [Citrullus lanatus var. lanatus (Thunbs) Matsum Nakai] because of its direct feeding and because it transmits viruses of important diseases. A series of studies were conducted for the development of native host plant resistance to whiteflies in cultivated watermelon. We have shown that a desert watermelon, Citrullus colocynthis (L.) Schrad, has resistance to whiteflies and to some other pests. Bioassays for native host plant resistance to whiteflies in C. colocynthesis accessions indicate that antibiosis, antixenosis and tolerance factors exert roles in the resistance. The bioassays identified differences among accessions within and among three selected species of Citrullus. Plant phenotype and leaf chemical profiles differ between accessions of C. colocynthis and watermelon cultivars. Moreover, plants of C. colocynthis accessions were noticeably odorous, and the total flavonoid content of their leaves was elevated as compared to watermelon cultivars. Genetic similarity and relationships were examined among accessions of Citrullus spp., Cucurbita maxima Duchesne (winter squash), Cucurbita pepo L. (squash and pumpkin), Cucumis melo L. (melon), Cucumis sativus L. (cucumber), and Lagenaria siceraria (Mol.) Standl (bottle gourd) showed that each is distinct with similar genetic distances among these species. Obtaining a good understanding of the genetics and mechanisms affecting native plant resistance of Citrullus is critical in the development of watermelon that is resistant to whiteflies.

Keywords:*Bemisia tabaci, whitefly, watermelon, Citrullus colocynthis, breeding, germplasm, host plant, resistance*

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Comprehensive field screenings for whorl- and ear-feeding insect resistance in corn germplasm lines

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A holistic approach to developing new corn germplasm that confers multiple insect resistance in various plant tissues at different growth stages was examined in two separate studies. The first study was the screening of eight corn germplasm lines for resistance whorl damage to fall armyworm [Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae)]. Four corn germplasm lines with known levels of resistance to root- and ear-feeding insects, and four germplasm entries with different levels of S. frugiperda resistance were evaluated in the study. Based on cluster analysis of S. frugiperda injury rating and predator survey data,'Mp708' and 'FAW7061' were the most resistant, whereas 'Ab24E' and 'EPM6' were the most susceptible to fall armyworm feeding. The western corn rootworm-resistant 'CRW3(S1)C6' showed resistance to S. frugiperda feeding. 'CRW3(S1)C6' and 'Ab24E' had the highest and lowest predator abundance, respectively. In the second study, 20 germplasm lines from the Germplasm Enhancement of Maize Program were examined. Four types of insect damage were assessed on corn ears. They were corn earworm [Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae)] damage, maize weevil [Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae)] -damaged kernels (%), stink bug-discolored kernels (%), and other chewing insect (e.g., sap beetles, chocolate milkworm, and pink scavenger caterpillar)-damaged kernels (%). Smut [Ustilago maydis (DC) Corda] infection rate, and husk tightness and extension were also assessed. Three of the 20 germplasm lines were identified as being multiple insect and smut resistant. Of the three lines, two were derived from 'DKXL370,' while the third line was derived from 'CUBA117.'

Keywords:*fall armyworm, predators, corn earworm, cluster analysis, principal components analysis*

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Constitutive and induced resistance to insects: Potential and limitations

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The physicochemical mechanisms of defense against herbivores are produced either constitutively and/or in response to plant damage. In addition, plants also release volatile compounds in response to herbivore damage that attract the natural enemies of the herbivores. Chemically induced expression systems or "gene switches" enable temporal, spatial, and quantitative control of genes introduced into plants or those that are already present in the plants to impart resistance to insect pests. A number of inducible genes have been identified in plants based on endogenous chemical signals such as phytohormones salicylic acid and jasmonic acid, response to insect and pathogen attack, or wounding. Effectiveness of the chemical injury inducers in providing resistance to various insect pests has also been demonstrated. Proteinase inhibitors and oxidative enzymes such as polyphenol oxidase, peroxidase, and lipoxygenase are active for 2 ? 3 weeks after induction in the damaged plants. Exogenous application jasmonic acid and salicylic acid has also been shown to induce resistance to several insect pests. Induced resistance, both in transgenic and non-transgenic plants offers a considerable promise to increase the levels of resistance to insect pests. However, the levels of constitutive and induced resistance quite often are not enough to exercise adequate control the target pests, and hence, HPR can be used in combination with other components of pest management to reduce pesticide use for sustainable crop production.

Keywords:*Host plant resistance constitutive, induced, pest management*

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Repellent response of female agromyzid flies to leafminer-infested bean plants

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Liriomyza sativae Blanchard, L. trifolii (Burgess) and L. huidobrensis (Blanchard) are three invasive leafminer species (Diptera: Agromyzidae) in China that have caused significant economic damage on vegetables and ornamental plants. In the current study, the repellent responses of female adults to leafminer-infested bean plants were examined with a Y-tube olfactometer. For both L. sativae and L. huidobrensis female adults, the repellent effect of the bean plants on which L. sativae had fed and oviposited was stronger than those on which L. huidobrensis had fed and oviposited. The repellent effect of the bean plants infested with L. sativae 2nd-instar larvae was stronger than those infested with L. huidobrensis 2nd-instar larvae. In a similar experiment using L. sativae and L. trifolii female adults, the repellent effect of the bean plants on which L. trifolii had fed and oviposited was stronger than those on which L. sativae had fed and oviposited. The repellent effect of the bean plants had infested with L. trifolii 2nd-instar larvae was stronger than those infested with L. sativae 2nd-instar larvae. The repellent effects of leafminer-infested bean plants on female adults was confirmed and is discussed in a context of ecological genetics of insect-plant interaction, interspecific competition and displacement of the leafmminers.

Keywords:*Repellent responses, Interspecific competition, Liriomyza sativae, L. trifolii, L. huidobrensis*

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Impact of Scion and Rootstock Combination on Pest populations on Cherry and Apple Plants

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To determine the impact of grafting on insect pest populations on fruit trees, the combination of rootstock and scion cultivars on root and leaf pests was examined. The scarab beetle larvae (Scarabaeoidea) were used to assess for the effect on root pest population on cherry, while a leafminer (Lithocolletis ringoniella) (Lepidoptera: Gracilariidae) population was used for leaf pest assessment on apple plants. The results showed that mahaleb (Prunus mahaleb. C 500) as rootstock of two cherry cultivars [i.e., seven-year-old sweet cherry (Prunus avium) and sour cherry (Cerasus valgaris)] had significantly different number of scarab beetle larvae (23.1 ± 1.73, and 8.00 ± 0.73, respectively) (P = 0.0001). In contrast, when the leaf-feeding L. ringoniella on four apple cultivars with the same rootstock were compared, significant difference was detected in both leafminer population and its damage. The rootstock was Xinjiang wild apple (Malus sieversii) for four popular commercial apple cultivars 'Gala,' 'Fuji,' 'Qinguan,' and 'Hongxin,' respectively. The number of L. ringoniella larvae was significantly (P = 0.0001) different among the four cultivars. Qinguan had the most [(59.10 ± 2.68) / 100 leaves], whereas Red Star had the least number of the larvae [(25.00 ± 1.98) / 100 leaves]. The most severe leaf damage by L. ringoniella was on Gala (10.01 ± 1.49%), the least damage was on Hongxin (1.56 ± 0.60%). Thus,optimized selection of rootstocks and scions with native insect resistance could be one of the effective tactics for the integrated pest management in fruit crop orchards.

Keywords:*scion and rootstock combination, native insect resistance, scarab beetle larvae, leafminer, cherry,*

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Resistance in Peach Rootstocks and Scions to Lesser Peachtree borer (Synanthedon pictipes, Lepidoptera: Sesiidae).

Ted Eric Cottrell[1], Tom Beckman[2], Dan Horton[3]
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The lesser peachtree borer, Synanthedon pictipes (Lepidoptera: Sesiidae), is of economic concern to peach, Prunus persica (L.) Batsch, production across the southeastern United States. Oviposition by S. pictipes was examined on field-grown Prunus scion and rootstock cultivars and two endemic Prunus spp. using limbs, not roots, in the laboratory. In this study, oviposition was compared on the peach scion `Harvester', peach rootstock `Guardian', plum × peach hybrid rootstock `MP-29', and the plum hybrid rootstock `Sharpe'. A significantly lower percentage of eggs occurred on limbs of Sharpe rootstock than other choices. A choice test using two endemic hosts, black cherry (P. serotina) and Chickasaw plum (P. angustifolia), along with Sharpe rootstock, found a lower percentage of eggs on limbs of Sharpe than either endemic host. Earlier research found these two endemic hosts of S. pictipes less preferable for oviposition than peach cultivars. However, when only limbs of Sharpe and a decoy were used, almost all eggs were laid on Sharpe. But when Harvester and Sharpe limbs were paired side by side, a higher percentage of eggs were found on the Harvester limb than on the Sharpe limb. Because S. pictipes attacks host trees above ground and Sharpe rootstock on grafted trees grows below ground, this rootstock might be a management option against the congeneric, root-attacking peachtree borer, S. exitiosa (Say).

Keywords:*Prunus persica, Rosaceae, cisgenic, oviposition*

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S1009TU11

Integrated Pest Management

Patterns of Host Adaptation in *Frankliniella occidentalis* among Vegetable Crops

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The current study examined the variation in life table characteristics, and physiological, biochemical, and molecular bases of western flower thrips , Frankliniella occidentalis (Pergande) host adaptation patterns. The main objective was to determine whether host availability affects insect preference or population diversion on different hosts throughout the field season of vegetable crop production. Three vegetable hosts were used in the study. They were bean, garlic and pepper. Host shift made F. occidentalis offspring displayed longer developmental time, lower survival rate, lower oviposition rate, especially at the F1 generation, but the reduction recovered in the following generations. Host-returning test showed that F. occidentalis biological characteristic recovered a little, but it still has significant difference with original host population and adaptive host population. hybridization made F. occidentalis populations between different hosts showed that, host preference and other biological characteristics were never as strong as its parental populations. Detoxification enzyme activity bioassays showed F. occidentalis could activate its detoxification enzymes to cope the allelochemicals in the new host, especially at the initial stage of host change test. The detoxification enzyme activity was the highest within 24 h. After six generations, the detoxification enzyme activity was relatively stabilized, but it was still higher than the thrips fed on the control. When the differentiation among 5 populations was examined using RAPD technique, the study revealed there was a significant differentiation in these host-specific populations. The ramifications of host shift of pests in integrated pest management on vegetable crops are also discussed.

Keywords:*Frankliniella occidentalis, host adaptation Patterns, biological characteristics*

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Variation of cross-resistance of Cry1Ab- and Cry1Ac-selected Asian corn borer to other Cry toxins

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One of many strategies proposed to delay pest resistance to Bt crops is multi-genes strategy which could be practiced through multiple toxins in sequence use and/or different crops as well as pyramiding of more than one toxin. However, one requirement for the multi-genes strategy to work is that those different toxins should be not affected by cross-resistance. Understanding the cross-resistance patters of Ostrinia furnacalis with resistance to cry toxins from Bacillus thuringensis is key to developing multi-genes strategy for delaying resistance to Bt maize. The susceptibility of F1 offspring of reciprocal crosses between Cry1Ab- and Cry1Ac-resistant strains (ACB-AbR and ACB-AcR) to Cry1Ab, Cry1Ac, Cry1F, and Cry1Ie toxins were evaluated. Resistance levels exceeded 35- and 74-fold at 50% lethal concentration of the ACB-AbR and ACB-AcR strains, respectively. In addition, it was significantly decreased in susceptibility to Cry1Ac and Cry1IF in ACB-AbR strain with cross-resistance levels of 61- and 18-fold. Meanwhile, it was decreased in susceptibility to Cry1Ab and Cry1IF in ACB-AcR strain with cross-resistance levels of 10- and 16-fold. Allelic complementation tests were complementary and suggested that ACB-AbR and ACB-AcR evolved resistance to Cry1Ab, Cry1Ac, and Cry1F at common loci. The patterns of cross-resistance suggest Cry1Ac share same binding sites with Cry1Ab, and Cry1F shares one or more receptor with Cry1Ab and Cry1Ac. In contrast, ACB-AbR and ACB-AcR as well as the offspring of their reciprocal crosses were equally susceptible to Cry1Ie suggested that Cry1Ie did not share binding sites with Cry1Ab and Cry1Ac on BBMV of Asian corn borer.

Keywords:*Ostrinia furnacalis, Cry toxin, resistance, cross-resistance, Lepidoptera, Crambidae*

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S1009TU13

Integrated Pest Management

Area-wide monitoring of resistance to *Bacillus thuringiensis* Cry1Ab maize in *Diatraea saccharalis*

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The sugarcane borer, Diatraea saccharalis (F.), is a major target of transgenic maize expressing Bacillus thuringiensis (Bt) proteins in South America and the mid-southern region of the United States. During 2004-2009, a total of 3107 feral individuals of D. saccharalis were collected from multiple locations in Louisiana, Texas, and Mississippi and examined for resistance to Cry1Ab maize using F1/F2 screens. Major resistance alleles to Cry1Ab maize in the populations sampled during 2004 and 2008 in Louisiana, and 2007 in Texas, and 2009 in Mississippi were rare. The frequency of major resistance alleles was estimated to be 0.0011 with a 95% CI of 0.0003-0.0024 for the Louisiana populations sampled from 2004-2008, <0.0016, with 95% probability, for the Texas populations, and <0.0061 for the Mississippi populations. However, resistance allele frequency for the populations sampled in 2009 in Louisiana increased significantly, reached 0.018 with a 95% CI of 0.008 to 0.03. Since 2010, transgenic maize expressing pyramided Bt genes has been planted in the U.S. mid-southern region and by 2011, pyramided Bt maize already replaced the Cry1Ab maize as the dominant Bt maize traits for managing lepidopteran pests including D. saccharalis. The timely switching of the single-gene Cry1Ab maize to the pyramided Bt maize should prevent further increase of Cry1Ab resistance allele frequency and thus ensure the continued success of Bt maize for managing D. saccharalis in the region.

Keywords:*GMO, maize, Cry1Ab, resistance monitoring, sugarcane borer, stalk borers*

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Moving forward: Trends and future prospects of biotechnology in transgenic crops for insect control

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Biotechnological innovations in transgenic insecticidal crops has commenced a new era of crop yield protection. This technology is well embraced by farmers because it is affordable, safe, convenient and highly efficacious. However, it still faces problems such as development of resistant populations, and shift of target pest insects. A successful IRM program requires multiple modes of action to effectively mitigate the risk of development of resistance. Availability of genes with new insecticidal spectrum is a key to combat outbreaks of newly emerged pest insects in time. With issues of negative phenotypes, not every insecticidal gene can be successfully expressed in crop plants and developed into a product. To date, Bacillus thuringiensis is still the only source of commercialized insect control trait genes. Several genes with insecticidal activities were discovered from those so-called non-Bt species. Yet, most of them encountered major expression obstacles in planta. None has been commercialized thus far. Recently there has been a rapid increase of products containing stacked traits generated by conventional breeding of different trait events, or by molecular stacking of multiple traits in a single transgene locus. Such a trend continues to push the limits of biotechnology, seed production logistics and product registration.

Keywords:*transgenic crops, insect control, Bacillus thuringiensis*

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S1010TU01

Integrated Pest Management

Evaluating the IPM fit of insecticides ? Sorting myth from reality

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Establishing the 'fit' of insecticides in IPM is crucial to optimise their use and maintain beneficial populations in the crop and landscape. To provide timely information to pest management practitioners we developed a simple approach that allows the IPM fit of insecticides to be evaluated and compared. Field plots of cotton are established in a replicated design. Sunflowers are planted between replicates to help reduce pesticide drift risks and provide a source of pests and beneficials. A range of new or candidate insecticides are applied 4 to 5 times at 10 d intervals and plots sampled for pests and beneficials pre-spray and twice between applications. Sampling includes; suction sampling for mobile arthropods, leaf washing for sedentary arthropods and visual checks for arthropods not well sampled by the former techniques. Data are analysed for treatment effects, and insecticides ranked according to the reduction in beneficial abundance, for each species or group, compared with the untreated control; VL (very low), less than 10%; L (low), 10-20%; M (moderate), 20-40%; H (high), 40-60%; VH (very high), > 60% and the risk of pest resurgence. Outcomes are then summarised in an easy-to-use look-up chart which is distributed to all pest managers and cotton growers. Meta-analysis of commercial crops managed with more selective insecticides showed they had higher beneficial populations, confirming the value of rankings generated using this method. Though there are many trade-offs with such an approach it has been valuable in increasing adoption of IPM principles in cotton.

Keywords:*cotton, pesticides, IPM*

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Fungal endophytes as insect management tools in cotton

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Plants commonly harbour a diversity of fungi as asymptomatic endophytes that can provide protection from a range of biotic and abiotic stressors. In order to better characterize the diversity and potential functions of fungal endophytes associated with cotton (Gossypium hirsutum), we surveyed the diversity of fungal endophytes in cultivated cotton across multiple sites in Texas, USA. We recovered a number of fungal endophytes that are known to act as antagonists against insects, plant pathogens, and nematode pests. In greenhouse trials to investigate effects on insect herbivores, we successfully inoculated cotton seedlings using seed treatments of a field-collected endophytic strain of Paecilomyces sp. and a commercially available strain of Beauveria bassiana. In both cases, cotton aphid (Aphis gossypii) reproduction was significantly reduced on endophytically-colonized plants versus uninoculated controls in greenhouse performance assays. An important consideration for the potential use of fungal endophytes in IPM is the potential for either positively or negatively correlated effects of endophytic colonization on plant performance. We found varying dose-dependent effects of both B. bassiana and Paecilomyces sp. endophytic colonization on total plant biomass, shoot and root length of endophyte-colonized versus uninoculated control cotton seedlings. Results of ongoing replicated field trials testing endophyte inoculation efficiency and protection from insect herbivores will be discussed.

Keywords:*entomopathogen, fungi, endophyte, microbial ecology, Aphis gossypii*

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S1010TU03

Integrated Pest Management S1010

Integrating natural enemies and semiochemicals in cultural control for an effective IPM

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Since their discovery, many semiochemicals (behavior modifying chemicals) have been identified and synthetized, and a number of techniques have been developed to use them in integrated pest management (IPM) programs against insect pests. In particular, the uses of semiochemicals in IPM to monitor insect pest populations and to interfere with their behavior preventing agricultural damage are widely adopted by the growers. In the same time, evidences that semiochemicals can also modify the behavior of insect natural enemies inspired researchers to explore the possibility to use semiochemicals to conserve and/or enhance the efficacy of natural enemies in cropping systems. However the use of semiochemicals integrated with natural enemies in IPM is still limited despite the fact that scientists have made important advances in recent years to elucidate semiochemical-natural enemies interactions in multitrophic contexts. Here we provide an overview of the IPM strategies integrating semiochemicals and natural enemies; furthermore, the factors that could be considered for more widely adoption of these strategies are discussed.

Keywords:*Hymenoptera, parasitoid, tri-trophic interactions, herbivore-induced plant volatiles*

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S1010TU04

Integrated Pest Management S1010

Dispersal of *Euschistus servus* (Heteroptera: Pentatomidae) in time and space

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Phytophagous stink bugs are one of the key agricultural pests of food and fiber production around the world. In the southeastern United States, Euschistus servus is one of the most common stink bug species found feeding on cereal grains, row crops, tree fruits, and vegetables. Studies were conducted to investigate population dynamics, population structure, and intercrop movement by E. servus in agricultural farmscapes. Research methods included weekly sweep net sampling, mark and capture, and dissection of adults to rate gonad development. Data show that this species is clearly bivoltine and utilizes a wide range of nonagronomic and agronomic hosts over the course of a single year. Adults overwinter from November through February before emerging and completing a generation on naturally occurring plants or winter wheat. First generation adults invade corn, peanut, cotton and eventually late season soybean where they oviposit resulting in the second generation that completes development in early October. Adults invade cotton starting when the plants began to bloom and continue moving into cotton through the bloom period. Intercrop flight distance was generally short (2.7 to 8.2 meters in 24 h), but was highly aggregated on the edges of fields. Results show that there are predictable patterns in population growth and dispersal into fields.

Keywords:*landscape ecology*

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Landscape-level cultural control as an arthropod pest management approach in Texas High Plains cotton agroecosystems

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A comprehensive cultural management study, integrating landscape characteristics potentially influencing crop and non-crop source-sink relationships, characterized Lygus hesperus seasonal population dynamics in the Texas High Plains. Supporting sinks and destroying refuges adjacent to the crop are effective for managing some arthropod pests. However, determining the propensity of host source or sink is challenging, especially for highly mobile and polyphagous pests such as Lygus hesperus. A four-year project involved surveying and sampling Lygus in several sub-regions of the southwestern United States, including the Texas High Plains. In Texas, geographic information of the landscape vegetation complex was compiled from a 150-km radius region. Fifty irrigated cotton fields, selected to represent regional cotton diversity, and up to six non-cotton insect habitats within a 3-km radius of each, were sampled via sweep-net for 10 weeks. Seasonal average Lygus abundance data were regressed with 27 field characteristics (variables). These included habitat land coverage, distance between cotton and non-cotton habitats, habitat heterogeneity index, and numerous environmental/ecological variables. A 10-parameter linear model explained 93% of data variation. Parameters contributing significantly to variation in Lygus abundance in cotton were corn and sunflower acreages, cotton distances from several non-cotton hosts, and habitat heterogeneity index. Field marking and capture (FMC) studies were conducted using protein markers and enzyme-linked immunosorbent assays to characterize Lygus intercrop movement behavior. FMC can be used to study the effects of various crop management practices on L. hesperus intercrop movement and may apply to other pests and cropping systems.

Keywords:*Cotton IPM, Landscape level IPM, Ecological pest management*

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Spatio-temporal dynamics of mirid bug *Creontiades biseratence* (Distant) in Bt cotton and other host crops: An intraguild assessment in the endemic area of an emerging pest

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From 2002 commercially Bt cotton cultivation started in India making it largest grower (110 lakh ha) in the world. The ruling events have Cry 1 Ac as single or stacked gene. The reduction of insecticides on Bt-cotton is presumed to have led to an enhanced infestation of mirid bugs, mealy bugs. Like in Australia, China and US mirid bugs are becoming key pests in India also with advent of Bt cottons. Since 2005 mirid bug, Creontiades biseratence (Distant) (Hemiptara : Miridae) has emerged as major sucking pest in South India particularly in Karnataka state. Its most aggressive impact is being experienced in Haveri. Acephate and Profenophos are chemical control options. In present study the seasonlong dynamics of mirid bug in Bt cotton as well as its alternate hosts has been studied across 13 locations in Haveri district to address an endemic area intraguild. The peak incidence of mirid bugs (390/100 squares) was in second fortnight of November though it could exists throughout season. Summer cultivation of cotton also had severe infestation offering a niche is main-season off period. The mean incidencein the district was 128.3±18.3 / 100 squares. The incidence was in severe form throughout district on cotton. The key alternate host crops were pigeonpea, sunflower, sorghum, safflower, maize, castor and amaranthus. Maximum incidence of mirids was on pigeonpea (14.0±3.5 bugs/pl) and on maize. The correlation was significant, positive (r= 0.78) between pigeonpea and cotton mirid incidence. Sunflower was related negatively (r= -0.44).In an intraguild pigeonpea and maize could

Keywords:*Mirid,Bt Cotton,Hosts.Intraguild*

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Area-wide pest management of sucking bugs in temperate climates benefits from a landscape perspective in managing vegetation: Lessons from field crops of the North American Great Plains

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Pest management in field crops may best be approached from an area-wide perspective because of low crop value and spatially-variable pest severity. This situation provides the opportunity (and the challenge) to consider: a) regional pest risk assessment, and b) pest management tactics applied regionally. In the North American Great Plains, area-wide strategies are based on host plant resistance, crop diversification, and association of crops with non-crop host plants. Producers use aphid-resistant wheat cultivars when available and apply this tactic regionally where aphids regularly occur. Application of crop diversification occurs less frequently on wheat, unless it is linked to water conservation. In cotton growing regions of the southern plains, a complex of sucking bugs occurs. Key species have known sensitivity to cotton spatially associated to varying degrees with weeds, neighboring crops, and other landscape features. Regional pest risk assessment can be derived from analysis of these associations. Across these examples, plant diversification (habitat heterogeneity) measurement is useful for regional pest risk assessment, but its active manipulation is often contingent on non-pest issues. In contrast, host plant resistance has a rich history in area-wide aphid control.

Keywords:*Area-wide pest management, Pest risk assessment, Habitat heterogeneity*

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S1011TU01

Integrated Pest Management

The contribution of IPM to food security in the developing world

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The recent spike in food prizes worldwide and resulting civil unrest and riots in many developing countries have been a strong reminder of the growing problem of food insecurity in many countries of the world. Several factors like growing populations in the south, stagnant yields of many important agricultural crops, increasing competition between agricultural and biofuel production for arable land, and the increasingly detrimental effects of global climate change on agricultural productivity, especially in the developing world, sparked some serious rethinking on the future of agriculture. Initially the emphasis was mainly on increasing agricultural productivity through improvement of germplasm and intensification, e.g. through mineral and organic fertilization. However, there are serious pitfalls of such a strategy, particularly with regard to the resilience and sustainability of such intense production systems. An alternative but also complementary approach is looking at loss prevention and loss reduction. Biotic constraints like pests and diseases are among the most important yield-limiting factors for agriculture worldwide, and particularly in tropical and subtropical environments. Approximately 30-40% of the potential agricultural pre- and post-harvest output in the developing world is lost to pest and diseases. In this symposium we are trying to establish if and how Integrated Pest Management (IPM) can contribute to alleviate food insecurity in the developing world.

Keywords:*Contribution of IPM to Food Security Developing World*

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Advance in integrated pest management of crops in China

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Integrated pest management (IPM) in crops is an important strategy for insect pest control, which plays important role for sustainable agriculture. In order to meet demand of crop production in China, Chinese government launches a series of scientific program for insect pest researches in recent years. In follow of these projects, China has established a national and province network which consists of scientist teams and special facilities for insect pest researches in universities and institutes. And many achievements on pest control such as methods and technologies on monitoring and forecasting for outbreaks of major pest insects, ecological management for insect population, biological control, chemical control, and the commercial use of transgenic insect-resistant crops, as well as IPM systems in cotton, rice, corn, wheat, vegetables and other crops, have been established in recent years. The rapid progress from modern sciences such as biological engineering and information technology is bring a new revolution for agriculture science, which is pushing IPM theory forward and giving a new opportunity for IPM application in China. Deployment of GPS, GIS and computer science in pest management is taking pest monitoring and forecast work to a high level. Commercialization of insect resistance genetically modified crops is presenting a great potential for regional control of insect pests. Researches on landscape biodiversity and conservation biological control provide an important idea for developing sustainable agriculture in China.

Keywords:*Agricultural insect pests, IPM, research progress, China*

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Field evaluation of trap capture based threshold for application of entomopathogenic fungi in management of thrips on French beans

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French beans (Phaseolus vulgaris L.) are an important high value crop in Kenya. Straightness and spotlessness are important quality attributes of French beans. Thrips infestation on French bean leads to spotting and malformations making them unsuitable for the market. Kenyan growers rely heavily on chemical insecticides for thrips control without consideration of the side effects and control costs. Thus, finding alternative control options based on timely intervention is important to minimize negative effects of chemical sprays. Studies were conducted over two cropping periods to evaluate trap capture based economic threshold for application of Metarhizium anisopliae (Metsch) for management of thrips on French beans. The application of M. anisopliae at thresholds of 300, 600 and 900 thrips per trap per week effectively reduced the thrips population as compared to untreated control. The number of sprays of M. anisopliae decreased with an increase in the threshold level. Control plots and plots treated with alpha cypermethrin had the highest population of F. occidentalis as compared to the fungus treatments. Highest yield of French bean resulted from fungus application at 300 thrips per trap per week and it was comparable to yields from weekly sprays of M. anisopliae and alpha cypermethrin. Thus fungus based thrips management at action threshold of 300 thrips/trap/week reduced thrips incidence, increased yield, reduced number of sprays upto 20% and resulted in the highest net income.

Keywords:*French beans, Thrips, Economic Thresholds, Metarhizium anisopliae*

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S1011TU04

Integrated Pest Management

Development and diffusion of a new IPM approach for potato in the Andean region of Peru

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Many insect pests have co-evolved with potato in the center of origin in the Andes of Peru. Farmers control infestations of Andean potato weevils (Premnotrypes suturicallus) by applying hazardous insecticides. While weevils are the major pest above 3800 m asl, in inner Andean valleys also occur potato tuber moths (Phthorimaea operculella, Symmetrischema tangolias) and occasionally flea beetles (Epitrix spp.). We developed IPM that consists of new technologies and strategies (i) to inhibit the migration of weevils by using plastic barriers; (ii) to scout the occurrence of flea beetles and eventually control by one single application of low-toxic insecticides, and (iii) to apply attract-and-kill to manage potato tuber moths. A talcum-B. thuringiensis (Btk) product and attract-and-kill are highly effective for IPM in potato stores. Further, we consider the recuperation of parasitoids, lost through the intensive use of pesticides. The new technologies are well perceived by agricultural extension workers and farmers. Organic growers, in particular, have shown immediate interest in adopting plastic barriers. Partnerships have been built up with the private sector to introduce and make available plastic in adequate sizes (40 cm wide) and quality, and to register and commercialize a low-cost biocontrol product based on Btk as well as attract-and-kill (AdiosMacho). Main limiting factor for implementing IPM is the heavy competition of agrochemicals to which farmers have easy access through a strong network of pesticide sellers. The role of the Peruvian government is important for the regulation of highly toxic pesticides and for the wider promotion of IPM.

Keywords:*IPM Peru*

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S1011TU05

Integrated Pest Management

Some factors affecting the productivity of maize in small scale farmers' fields in Ekpoma, Edo State, Nigeria

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Some factors affecting the productivity of maize, Zea mays L., were investigated in resource poor farmers' fields in Ekpoma, Edo State, Nigeria, during the 2011 first and second planting seasons. Maize stand loss was more severe (42.83% t0 60.34%) between 11 and 13 weeks after emergence (WAE). Damage by stem borers was very low until 11 WAE (14.35%) and reduced to 4.43% at 13 WAE. Termites' damage increased from 35.44% to 56.96% (2 to 4 WAE) and decreased sharply, 16.24% to 0.00% (5 to 13 WAE). Defoliation was highest (49.58% to 80.38%) at 2 to 5 WAE, but wilting was more severe (15.40% to 28.69%) at 9 to 13 WAE. Significant positive correlations were found between the number of stands and defoliation ($r = 0.7544$; $P < 0.0001$) as well as dmage by termites ($r = 0.5108$; $P < 0.01$). Damage by stem borers was not significantly different between the seasons, but cob tip damage by earworms was significantly higher during the first season (0.84%), compared with the second season (0.30%). There was a significant negative correlation ($r = -0.4519$; $P < 0.01$) between the length of the sheath of the flag leaf and damage to the tip of the cob. The weight and length of the cobs had significant positive correlations with the length of the flag leaf and the entire maize stalk. The implications of these results in the development of effective insect pest management strategies to improve maize production among resource poor farmers are discussed.

Keywords:*Maize, stem borers, termites, earworms*

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S1011TU06

Integrated Pest Management

How the use of integrated pest management for vegetables contributes to food and nutritional security in Asia

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Approximately 85% of global vegetable production occurs in Asia, on about 78% of global vegetable area. Pests and diseases constrain vegetable production in tropical Asia and growers rely heavily on chemical pesticides for control. Pesticide application often exceeds 50 sprays per crop season on eggplant in the Philippines or tomato in south India. Indiscriminate use of pesticides leads to the development of resistance in major insect pests including tomato fruitworm (Helicoverpa armigera), eggplant fruit and shoot borer (Leucinodes orbonalis), diamondback moth (Plutella xylostella), and whitefly (Bemisia tabaci). Overuse of pesticides degrades the environment and causes resurgence of secondary pests. Alternative integrated pest management (IPM) strategies are warranted to reduce pesticide residue on vegetables and in the environment, and to protect human health. For four decades, AVRDC ? The World Vegetable Center has developed and promoted effective vegetable IPM strategies to manage insect pests on brassicas, eggplant, vegetable legumes and tomato in tropical Asia. These strategies have decreased pesticide misuse and enhanced the income and livelihoods of people throughout the region. With the introduction of biological control of P. xylostella in Southeast Asia, the income of growers doubled due to reduction in spraying costs and increased productivity. In South Asia, eggplant IPM adopters increased their profit margins and their crop acreage. The per capita availability of vegetables has almost doubled in Asia over the last two decades, in part due to IPM; people now have greater scope to improve their diets with a variety of nutritious and health-promoting vegetables.

Keywords:*Vegetable IPM, Asia, Pesticide reduction, Enhanced Productivity, Diet diversity*

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S1011TU07

Integrated Pest Management

Push-Pull: A platform technology for enhancing food security and environmental sustainability in Africa

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The Push-Pull technology is based on a novel cropping system developed for integrated pest, weed and soil management in cereal-livestock farming systems. The system makes use of two kinds of companion plants, a trap crop that serves as an attractant for stem borers, to pull them away from cereal field, and a second crop intercropped within the rows of cereal crop to repel the pests. The most effective trap crop, Napier grass, is planted as a border around the main crop. The fodder legumes in the genus Desmodium act as a repellents for stemborers with the added benefit of fixing nitrogen in the soils as well as serving as a cover crop to prevent soil erosion. The root exudates of Desmodium spp. contain novel flavonoid and isoflavonoid compounds that interfere with striga parasitisation of cereals. Both Napier grass and Desmodium plants provide high value animal fodder, facilitating milk production and diversifying farmers' income sources. The technology is appropriate to smallholder farmers practicing mixed cropping systems in Africa. It effectively addresses major production constraints, and increases cereal yields (eg, maize yields from below 1 to 3.5t/ha). The technology is economical as it is based on locally available plants and not expensive external inputs. Adopted by over 47,000 farmers to date in East Africa, key factors in its further up-scaling include effective technology dissemination, adaptability of companion plants for climate resilience, capacity building, multi-stakeholder collaboration, integration with livestock husbandry, improvement in Desmodium seed accessibility and creation of a supportive policy framework.

Keywords:*Habitat Management, Biological Control*

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Integrated Pest Management

Success and challenges in integrated management of cassava and plantain pests in sub-Saharan Africa

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Pests and diseases continue to be the main constraints of cassava and plantains, two major staple crops in sub-Saharan Africa. Biological control has been successful against exotic pests - cassava green mite and cassava mealybug, but not against indigenous pests ? the whitefly Bemisia tabaci and the scale Stictococcus vayssierei. Similarly, the main plantain pests, the weevil Cosompoletis sordidus and the aphid Pentalonia nigronervosa - vector of banana bunchy top virus have required complicated integrated solutions. A combination of biological control and host plant resistance are presently being explored for the management of Bemisia tabaci. For the management of S. vayssierei, a combination of tolerant cassava genotypes, ant control and pre-planting host residue removal have been developed, but the implementation of these options ? other than tolerant varieties ? continue to be quite challenging. Cosmopoletis sordidus has received a great deal of research which has resulted in the development of integrated options using host plant tolerance, biological and microbial control (including endophytes) and cultural management. Although successful in weevil suppression, these have not been widely implemented and adopted. Recent research on the duo of the aphid P. nigronervosa and banana bunchy top disease (BBTD) has not identified any effective natural enemies of the aphid but limited success has been achieved in identifying plantain genotypes with reduced susceptibility to the aphid and BBTD, which are now being explored in combination of sanitation options in the management of banana aphid and BBTD.

Keywords:*Bemisia tabaci, Cosmopoletis sordidus, Pentalonia nigronervosa, Stictococcus vayssierei, banana bunchy top disease*

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S1011TU09

Integrated Pest Management

IPM in date palms and its contribution to food security in the Middle East

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The date palm, Phoenix dactylifera, is considered one of the oldest fruit trees, which is mentioned in the Qur'an and Bible. There are 100 million date palms worldwide, of which 62 million palms can be found in the Middle East, which are the major countries to produce dates that are most used by Middle East people as food security. Currently, 26 palm species are reported as hosts of the red palm weevil (RPW), Rhynchophorus ferrugineus. In the mid-1980's, it entered in the date plantations of the arid Middle-Eastern region from where it moved into Africa (Egypt) in the early 1990's and subsequently into Europe (Spain). It is devastating Phoenix canariensis in the Mediterranean basin. In 2009, it gained entry into the Caribbean (Curacao Island). In 2010, it was detected into Lebanon and Laguna Beach, California and in Dec. 2011 in Carthage, Tunisia. RPW mostly infests young palms less than 20 years old with a single female laying about 300 eggs in its lifespan. Palms in the early stage of attack are difficult to detect while infested palms in the late stage of attack harbor several overlapping generations that exhibit extensive tissue damage. Such severely infested palms do not respond to curative treatment and have to be eradicated. RPW is currently managed through a pheromone based Integrated Pest Management (IPM) strategy where early detection of infestation is the key to ensure success. Implementing of quarantine regulations is essential to curtail the spread of this pest.

Keywords:*Date palm, Phoenix dactylifera, Red palm weevil, Rhynchophorus ferrugineus, IPM*

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S1011TU10

Integrated Pest Management

IPM in rice - Has it contributed to food security in Asia?

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Rice IPM became prominent in 1986 when Indonesia announced a ban of 56 insecticides, removed the subsidy and introduced IPM field schools. Since then millions have been spent and about a million farmers have "graduated" from IPM-FFS. This was instituted when Indonesia and many Asian countries were experiencing massive destructions by the brown planthopper (BPH). Thirty years later, rice production in Asia is experiencing far greater and persistent threats from the BPH and virus diseases than ever before. Thailand is losing rice to BPH for 9 consecutive seasons. In 2010 Thailand rice production lost 1.1 million tons of paddy and in 2012, a loss of 500,000 tons is expected.. Similarly Indonesia is suffering the same threats and had lost about a million tons. BPH is an insecticide induced secondary pest and outbreaks are due to mismanagement that destroys ecosystem services. We now need to ask the hard questions "Has IPM really contributed to rice food security?". If IPM had been successful, why is it that we now face the same problem that IPM was meant to fix? Why does this problem continue to haunt and threaten rice production in Asia? The presentation looks at the current state of insect management in rice, BPH outbreak threats, factors that might have contributed to eroding the gains from IPM and explores for a broader framework for improvements. It will discuss structural and policy issues that underpin the "wicked problem" of insecticide use, BPH outbreaks and the prospects of using ecological engineering approaches.

Keywords:*IPM, ecological engineering, insecticide misuse, BPH*

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S1012TU01

Integrated Pest Management

Innovative agro-ecological strategies for management of cereal stem borers

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The basic foundation of successful IPM implementation is a thorough knowledge of the target pest's life cycle, and its ecological and behavioral interactions with the environment and natural controlling factors in crop and especially indigenous habitats followed with sound technology transfer to target farmers. Increased species diversity in and around cropping systems and structured habitat management systems are becoming more important as IPM tools. These tools include agro-ecosystem management to make the crop less suitable for pest colonization, and to increase natural enemy foraging and abundance in the crop habitat, thereby increasing efficacy of conservation, inoculative and augmentative biological control. A novel habitat management strategy, using several components of the push-pull approach developed in east Africa, has been developed for Eldana saccharina (Lepidoptera: Pyralidae) in South African sugarcane. In this strategy indigenous host plants of E. saccharina, for example Cyperus dives (Cyperaceae) are used as 'pull' or attractive plants, while Melinis minutiflora (Poaceae) functions as a 'push' or repellent plant for the moths. Genetically modified Bt maize producing a toxin lethal to E. saccharina larvae, is used as a dead-end trap crop in the system. Increased biodiversity in agro-ecosystems generally leads to efficient natural control of crop pests and diseases. Need assessments and good technology transfer complements successful IPM adoption.

Keywords:*Pest management, stem borers*

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Key agronomic and ecological factors impacting moth borers populations and their natural enemies in the context of sugarcane expansion and intensification.

Francois-Regis Goebel
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Global intensification and expansion of agricultural areas for more food and energy products is expected to change pest-plant interactions and the related ecosystem services. For example, intensification of agronomic practices, land use change, landscape fragmentation, incorporation of crop genetic improvements and altered harvest regimes lead to predictable shifts in pest and beneficial natural enemy communities in new sugarcane areas. This is particularly the case in the developing countries where natural vegetation patches are rapidly converted into agricultural land. In this context, the risk of biodiversity loss associated with pest outbreaks is often mentioned. Through different experiences in several sugarcane producing countries, it is shown how over application of fertilizers, water stress, misuse of insecticides, harvest delay, trash blanketing cane burning, use of susceptible varieties, Silicon deficient soils can dramatically increased infestation from major moth borers such as Eldana saccharina (Lepidoptera: Pyralidae) and Chilo sacchariphagus (Lepidoptera: Crambidae) with sometimes severe yield losses. This situation is often exacerbated by the lack of natural enemies, particularly in large-scale monoculture systems where natural vegetation has been cleared. The impact of such factors on arthropod communities should be carefully analyzed and incorporated in a new ecologically-based pest management approach at a landscape scale. This calls for increased scientific understanding of landscape effects on spatial crop?pest?natural enemy interactions and their ecosystem services such as biocontrol in the sugarcane areas.

Keywords:*stemborers, sugarcane, infestation, agricultural practices, ecosystem services, beneficials*

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S1012TU03

Integrated Pest Management

Bio-intensive integrated pest management of invasive alien plants and indigenous insect pests of Graminaceous crops in a single programme: Fact or fantasy?

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Bio-intensive integrated pest management (IPM) is based on holistic agro-ecosystem interactions, in which knowledge about insects, their symbionts, pathogens, natural enemies, plants, endophytes and interactions between these are combined to develop IPM in an area-wide (AW), sustainable manner. Habitat management is advocated as part of AW-IPM by influencing behaviour of natural enemies and their hosts in such a way as to increase effectiveness of natural enemies in a crop. This paper proposes that biological control of invasive alien plants (IAP's) can contribute to habitat management as part of a bio-intensive AW-IPM for an indigenous stemborer, and that knowledge of the chemical ecology of the agro-ecosystem will enhance AW-IPM. Eldana saccharina (Lepidoptera: Pyralidae) is native to wetland plants in Africa. Its wetland habitat has been encroached (and surrounded) by crops providing food, and IAP's such as Eichornia crassipes, Pistia stratiotes, Lantana camara and Chromolaena odorata. These are generally more competitive than the indigenous wetland sedges that provide stable habitats for this borer and its natural enemies. It will be shown that augmentative mass releases of classical biocontrol agents of IAP's reduce the cover of these, providing opportunity for re-establishment of indigenous wetland plants, and thus habitat for indigenous natural enemy/host interactions, and how these will help manage pest populations in adjacent crop habitats. Furthermore, the complex nature of host location by parasitoids using volatiles emitted from indigenous compared to crop hosts in these agro?ecosystems will demonstrate the importance of a knowledge of chemical ecology in any bio-intensive AW-IPM strategy.

Keywords:*Chemical ecology, biological control, Habitat management,agro-ecosytems*

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Sterile insect technique in sugarcane pest management

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Stemborers are constraints to world sugarcane production. Conventional control and integrated pest management (IPM) measures have worked in certain countries, but in others stemborers remain problematic and biosecurity risks. A bio-intensive control approach is advocated, based on holistic agro-ecosystem interactions, to develop IPM in an area-wide (AW) sustainable manner against these. Included here is Sterile Insect Technique (SIT). Eldana saccharina (Lepidoptera: Pyralidae), severely limits sugarcane production in South Africa. This paper shows SIT as a viable control option. Calco Red N1700, fed to E. saccharina larvae in a diet, coloured adult fat bodies red, with no ill-effects on development and reproductive biology. They could thus be separated from field adults. Radiation biology studies showed that F1 male sterility was attained by exposing "fathers" to 200 Gy. Minimal emergence from F1 progeny of males exposed to 150Gy makes this radiation dose an alternative. Males irradiated at 150 and 200 Gy commenced calling at the same time as untreated laboratory reared males, and at least six hours before field males. Similarly, irradiated males commenced mating with field females within 30 minutes of calling initiation, and much earlier than wild males. They mated with three to five times more wild females than wild males, and remained in copula with wild females for an hour longer than wild males. In choice experiments irradiated males were as competitive at attracting and mating with wild females as wild males were. SIT, using F1 sterility, can thus be developed as part of AW-IPM against E. saccharina

Keywords:*F1 Sterility, Radiation biology, stemborers, mating studies*

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Integrated pest management of sugarcane moth borers: An Indonesian experience

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An Integrated Pest Management program is developed to combat moth borer infestation in Java, Indonesia. An extensive survey over a period of two years (2010 ? 2011) revealed the existence of five moth borer species causing damage to sugarcane crops in Java, and these were Chilo auricilius, C. sacchariphagus, Scirpophaga excerptalis, Sesamia inferens and Tetramoera schistaceana, with the three former species being the most abundant. Infestation by C. sacchariphagus and C. auricilius caused dead heart and leaf damage in young cane and bored internodes in older cane, while S. excerptalis was responsible for the majority of dead heart symptoms at all stages of plant development. Infestation by the main three borers commenced in February and escalated to a peak in about June ? July. Our results revealed very low levels of parasitism by key natural enemies, and this was attributed to inconsistent parasitoid release, lack of prior assessment of infestation levels, release of low parasitoid densities and random use of insecticides. Certain varieties demonstrated a degree of tolerance to borer infestation, however, different susceptibility levels to different borer species was evident, hence, breeding for combined resistance to all borer species is difficult. A plan is developed based on borer abundance in the field, whereby accurate densities of natural enemies are released as borer infestation commences and well before it reaches a peak. The principal components of a detailed Integrated Management Program to combat moth borer infestations in Indonesia are discussed.

Keywords:*moth borer, Indonesia, Chilo, Scirpophaga*

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Varietal resistance to stemborers in Papua New Guinea

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Commercial crops of sugarcane at Ramu Agri-Industries, Papua New Guinea, are attacked by several species of moth borers, mainly Sesamia grisescens, Chilo terrenellus and Scirpophaga excerptalis, as well as the sugarcane weevil borer Rhabdoscelus obscurus. We analysed the results of variety trials planted in 2003, 2007, 2010 and 2011 with the aim of evaluating varietal responses to these borers, and particularly to S. grisescens. For all species, damage and/or numbers of insects differed significantly among clones in at least one trial. Levels of internode damage by S. grisescens to 12 standard clones, chosen to represent a range of responses to that borer, were correlated between most of the trials and crop classes. There were correlations, both positive and negative, between damage from the different borers. Cane yields, where measured, were negatively correlated with damage from S. grisescens but not C. terrenellus or S. excerptalis. These results confirm the importance of S. grisescens as a major pest of sugarcane in PNG, as well as the existence of resistance to this pest among sugarcane clones. In 2011, we also began artificial infestation experiments using eggs of S. grisescens in an attempt to develop a rapid screening method for resistance to this pest without interference from the other species. However, infestation procedures have so far proved unsatisfactory.

Keywords:*Moth borer, weevil borer, Sesamia grisescens, Chilo terrenellus, Scirpophaga excerptalis*

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Significant improvement of IPM strategies against the sugarcane stem borer, *Chilo sacchariphagus*, through long-term succesful storage of *Trichogramma chilonis* (Hymenoptera:Tric)

Hong Do Thi Khanh[1], Adrien Bonnet[2], Johanna Bodendorfer[3], Etty Colombel[4], Francois-Regis Goebel[5], Marlene Marquier[6], Clarisse Clain[7], Estelle Roux[8], Elisabeth Tabone[9]

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The stem borer Chilo sacchariphagus is a key pest of sugarcane in Reunion Island and South-East Asia. Since 2002, an IPM strategy associating inundative release of an egg parasitoid, Trichogramma chilonis, and conservation of a predatory ant has been proposed. This reduced up to 50% of damage in field experiments in Reunion Island (financial gains from $800 to $1800/ha). To develop IPM strategies on a large scale, reducing the global costs is necessary. In this context, cold storage is a key step to the development of the sugarcane borer biocontrol, by improving production and release management. Thanks to a French funding (CASDAR), laboratory tests on cold storage of T. chilonis were first conducted, followed by field experiments on the performance of stored parasitoids. Quality control after storage was performed several times (after mass production, before and after transport, after field release) in laboratory at 25 ± 1°C, RH 75 ± 5 % and 16L: 8hD to evaluate emergence rate of stored insects, fecundity during 7 days and survival at 7th day of T. chilonis females. For the first time, a long-term cold storage of T. chilonis was obtained without loss of physiological performance (good emergence, fecundity, survival during 7 days). Results suggested a quiescence state because no loss was observed due to storage interruption. Moreover, the same field efficacy of cold stored Trichogramma compared to non-stored individuals was obtained in Reunion Island. These important results make possible a large-scale application of IPM strategies in sugarcane production.

Keywords:*Chilo sacchariphagus, Trichogramma chilonis, inundative release,cold storage, diapause, quiescence, mass production*

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Key steps of a successful biological control against the sugarcane stemborer by inundative release of *Trichogramma* spp.

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For over ten years, a biological control strategy by inundative release of Trichogramma chilonis (Hymenoptera: Trichogrammatidae) against Chilo sacchariphagus (Lepidoptera: Crambidae) has been studied (French collaboration between INRA, CIRAD and FDGDON Reunion Island). Many key steps were elaborated the last years regarding the pest and beneficial insects. Strategies of biological control, mass production, and field releases were also investigated. Important results were obtained such as: (i) population dynamic of Chilo sacchariphagus and damage, (ii) efficacy of the egg parasitoid Trichogramma chilonis, (iii) strategies of insect release (period, dose, frequency, adapted packaging, and delayed emergence), (iv) optimization of mass production, (v) cold storage, (vi) field release methods and checking of field efficacy. An IPM strategy associating inundative release of an egg parasitoid, T. chilonis and conservation of a predatory ant, Pheidole megacephala, has been proposed. According to field experiments in Reunion Island, this reduced up to 50% of damage with financial gains ranging from 800 to 1800 dollars per ha. Successful long-term storage of T. chilonis and its good efficacy in field after cold storage make possible a large-scale application of IPM strategies in sugarcane production.

Keywords:*Chilo sacchariphagus, Trichogramma chilonis, cold storage, diapause, quiescence, mass production, sugarcane, Lepidoptera*

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IPM module for the sustainable management of sugarcane stem borer, *Chilo infuscatellus* (Pyralidae , Lepidoptera) in Pakistan

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The present study describes the evaluation of some environmentally sound tactics towards the management of sugarcane stem borer (Chilo infuscatellus). Different approaches were applied, Trichogramma cards, Detrashing, Light traps and Chemical control. The effects of these were found on the basis of incidence and intensity of Chilo infuscatellus. It was concluded from the results that the minimum infestation (8.71%) was recorded in T7 (Trichogramma cards + Detrashing + Light traps) which was statically at par with those of T3(Trichogramma cards + Light Traps), T1(Trichogramma cards + Detrashing) and T4(Detrashing + Chemical control) with increase in percent infestation 8.92%, 9.34% and 9.74% respectively. The treatment T8(Trichogramma cards + Chemical control + Light traps) showed 10.46% infestation was significant different from T6(Trichogramma cards + Detrashing + Chemical control), T9(Chemical control + Light trap + Detrashing) with infestation 11.66% and 11.86%. The treatment T2 (Trichogramma cards + Chemical control) with infestation 12.39% was statically at par with those of T5(Detrashing + light traps) with infestation (12.82%). The maximum infestation was observed in control treatment with 25.45%. The minimum infestation (11.66%) was recorded after 10 days interval while maximum infestation (12.53%) was observed after 20 days interval.

Keywords:*IPM, Sugarcane Stem Borer, Chilo infuscatellus, Trichogramma, Light Traps*

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Study of adaptive odor searching behavior using a brain-machine hybrid system

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Insects perform adaptive odor searching behavior by integrating multimodal information. This adaptive behavior is generated through the interaction among a brain, a body and environments. To examine the adaptive processing of an insect's brain, we developed a brain-machine hybrid system which is a mobile robot mounted a male silkworm moth's head as a controller and sensors. Male moths orient toward female moths detecting upon conspecific female moths' sex pheromone. The robot was controlled according to the steering signals recorded bilaterally from neck motor neurons, 2nd CNb (2nd cervical nerve b) connecting with the pre-motor areas in the brain. We set the spike-behavior conversion rule that the spiking rates were linearly translated into the robot's wheels rotation. We could observe the moths' programmed behavioral pattern and orientation behavior on this system. Using this system, we examined whether moths could modify command signals responding to the given disturbances or changes of behavioral parameters. As a result, moths responded to the given angular velocity by using visual feedback. Moreover, when we biased left and right motor outputs and doubled angular velocity during odor source localization, activities of the 2nd CNbs were modulated depending on the motor parameters, and in several examples the hybrid system still could reach the pheromone source. From the results, moths on the hybrid system could show compensative responses to keep its angular velocity. I will discuss how moths generate this adaptive behavior using olfactory and visual information.

Keywords:*Brain-machine hybrid system, Adaptive behavior, Chemical plume tracing, Moth*

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Chemical plume tracing performance depending on angular velocity observed by a brain-machine hybrid system

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Silkworm moths exhibit adaptive behavior according to dynamic environments during the chemical plume tracing (CPT). The purpose of this study is to investigate whether the adaptability contributes to the CPT performance. We consider the adaptability should be implemented in order to realize an effective artificial CPT system. To investigate the moth's brain functions to modify its behavior, we identified the brain as a linear dynamic model by using a brain-machine hybrid system, which was composed of a moth's brain and a robotic body. The system enables us to investigate how the brain adapts to the changes of the body properties. In the experiment, we measured the neural responses to rotational disturbances to the system by changing the motor gain of the robot. Regarding the disturbances as input and the angular velocity which were calculated from the neural responses as output, we identified the transfer function. Comparing the two transfer functions obtained by changing the motor gain of the robot, we found that the parameters of the transfer functions were kept constant regardless of the motor gain. In short, the brain has the ability to keep the angular velocity responses constant. Then we evaluated by computer simulations whether the CPT performance was related to the angular velocity. As the results, we obtained the optimal value of the angular velocity during the CPT. In conclusion, adjusting the angular velocity of a robot to the average angular velocity of moths potentially improve the CPT performance.

Keywords:*Chemical plume tracing, Moths, Adaptability, Brain-machine hybrid system*

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Extraction of morphological characteristics of insect specimens for automatic identification by using wavelets and self-organized map

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Automatic identification of specimens is prerequisite in monitoring and guaranteeing reliable measurements of species composition in ecosystems. Wavelets and Self-Organizing Maps (SOMs) were utilized to extract the colored wing patterns of insect species for automatic identification in this study. Species in different families including Papilionidae and Pieridae in Lepidoptera were selected, and the images were accordingly pre-treated to minimize individual variation and noise. Wavelets were subsequently applied to feature extraction of the colored patterns. Wavelet coefficients were accordingly calculated based on the basis function (e.g., Morlet). The obtained coefficients were subsequently provided to Self-Organizing Map (SOM) for classification of the specimens. Although individual variation was observed, the combinational use of Wavelets and SOM was efficient in characterization of the colored patterns and identification of the insect specimens. Application of computational methods to automatic detection of specimens was further discussed regarding objective species assessment in ecological sciences.

Keywords:*Wavelet, SOM, Lepidoptera, Image Processing, Species Identification*

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S1013W04

Integrated Pest Management

Agricultural robot for pest management and in situ pest recognition

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Pest damage is one of the main factors to cause severe production loss in agriculture. In this study, a greenhouse robot is developed for controlling pests with minimal dose of pesticides and for automatically detecting pest distribution in greenhouse. The 3 degree of freedom manipulator of the robot ensures the precise spray to individual leaves with minimal volume of chemicals. A sampling device (e.g, sticky trap) is additionally installed on the robot for attracting pests. The images of pests attracted to the sticky traps were analyzed by multifractal dimension. The tiny size of pests (i.e., whitefly) is efficiently recognized to be able to count the number of insects attached to the traps in unit time. As the robot patrols the greenhouse, the pest detection data are recorded concurrently, providing information on spatial distribution as the time progresses. The in situ pest distribution and pest dispersal were further discussed regarding prediction of pest density and pest control strategy.

Keywords:*pattern recognition, precision pest control, automatic sampling, greenhouse management*

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Recognition of the line-shaped movement of aquatic macroinvertebrates in stressful conditions

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Aquatic macroinvertebrates are considered as an efficient indicating taxon in response to environmental variability. Recently response behaviors have been regarded as an effective tool for monitoring disturbing agents in aquatic ecosystems. Behavior of individuals could change instantly in time (e.g., changing shape, speed, acceleration, turning angles) and could efficiently present the time progress of response behaviors to disturbances in situ. Most studies, however, have been conducted with the point data to indicate movement positions of the round shaped indicator species. In this study, behaviors of the line shaped species (e.g., chironomid larvae) were analyzed with the movement segments continuously recorded by the video-tracking system. The body components were analyzed before and after the treatments of toxic chemicals (including formaldehyde). Different behavior responses were detected after the toxin treatment along with time progress (e.g., irregular movement, shaking). Behaviors of chironomid were compared with behavior factors of other line-shaped organisms (e.g., C. elegans). The study on the line-shape movement would contribute to broadening the scope of behavior monitoring and would be suitable in development of early-warning system to detect disturbances in environment.

Keywords:*behavior response, biological detection, indicating behavior, tolerance, movement tracking*

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Analysis of movement behaviors in response to chemical stressors using self-organizing map and hidden Markov model

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Movement tracks of indicator species, including Drosophila melanogaster, were continuously observed before and after the treatments of toxic chemicals (formaldehyde) at different concentrations (0.1, 1.0 and 2.5 ppm) based on computer vision techniques. The positional order of the movement was classified into different patterns in two dimensions. The Self-organizing map (SOM) was applied to classification of movement behaviors before and after treatment. Subsequently, a hidden Markov model (HMM) was utilized to sequence changes in behavior states of indicator species. The transition probability matrix (TPM) and the emission probability matrix (EPM) were accordingly obtained through HMM. Efficiency of HMM was further discussed in behavior monitoring effects of disturbances in environment.

Keywords:*Permutation Tests, behavior analysis, behavior observation, behavior response*

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Analysis of gene information of insects based on pattern recognition techniques

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The DNA sequence and amino acid data of insects including Chironomide (Diptera) were analyzed by Self-organizing map (SOM). Abundance distributions of genotypes at DNA and amino acids levels were further compared in different species according to different patterns obtained by the SOM. The SOM patterns accordingly characterized the species clusters, reflecting ecological (e.g., feeding types) and geographical (e.g., distribution) effects. The relationships between DNA sequence and amino acids were computationally addressed to reveal structural properties residing in hierarchy of DNA and amino acid. The models for abundance distribution patterns including log-normal distribution were obtained according to different taxa, and accordingly expressed the complexity of gene information. By distinguishing the differences at the layer of DNA sequences and amino acids, evolution and adaptation of the species could be efficiently addressed in response to environmental constraints.

Keywords:*self-organizing map, gene abundance distribution, DNA and amino acid sequence analyses, genetic dist*

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Wavelet method applied to analysis of movement behavior of indicator species

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We analyzed the time development of movement behavior of indicator species using the wavelet method. Movement of indicator species (Daphnia magna) was continuously recorded and the movement tracks were transformed to time series of coordinates. We analyzed the time series of movement track data using the wavelet method and subsequently introduced quantum mechanical analogy to quantify the movement tracks. Each mode in the wavelet method was considered as a quantum mechanical state while the amplitude of each mode as the probability. The quantum mechanical entropy, von Neumann entropy, could be evaluated and the entropy was compared with others including Shannon entropy and Permutation entropy. We also discussed about application of the method for monitoring chemical stress in response behaviors of the indicator species.

Keywords:*monitoring, quantum mechanical analogy, von Neumann entropy, Shanon entropy, Daphnia*

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S1013W09

Integrated Pest Management

S1013

Computational methods for analyzing morphology and behavior of macroinvertebrates and insects for monitoring

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Automatic image processing has been developed rapidly for monitoring in various fields since late 1990's, including pest management, species diversity maintenance, and risk assessment. Recent development of computation models are outlined in detection of insects and macroinvertebrates and in tracking movement behaviors under various environmental conditions. In addition to statistical analyses, the methods include information extraction, patterning, stochastic processes, and time series analyses. For information extraction entropy (e.g., permutation entropy) and multi-fractal dimension were used to express the structure property residing in the behavioral data. Ecological informatics including self-organizing map and multi-layer perceptron network and wavelets were applied to patterning the complex data under stressful conditions. For elucidating stochastic processes in behavioral changes, hidden Markov models were implemented to address transition probability in behavioral changes. For time series analyses temporal networks such as recurrent neural network and cross-correlation analyses were used for predicting time delay effects of behavioral changes. Efficiency of in situ behavioral monitoring was further discussed for application including pro-ecological pest control and conservation management.

Keywords:*insect recognition, behavior analysis, warning system, informatics, hidden Markov model, time-series*

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Range expansion of the southern green stink bug *Nezara viridula* in Japan with advance in the climate warming

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The impact of climate warming on insects was studied using the southern green stink bug Nezara viridula which is a tropical species in its origin. At first, past and current limits of the distribution range of this species were compared. As a result, it was found that the climate warming promoted northward range expansion of Nezara viridula by providing favourable overwintering conditions close to the specie's range limit. At the same time, the life history traits such as an intensity of reproductive diapause is on the way of changing with northward range expansion. Furthermore, this range expansion of Nezara viridula is now accelerating the extinction of temperate closely related species, Nezara antennata, by the infertile interspecific copulation. The progeny of field collected adults was reared under outdoor conditions and under virtual climate warming conditions (incubator which simulated climate warming by adding in real time 2.5℃ to the outdoor conditions). Even such a moderate temperature increase affected many life history traits of Nezara viridula. The warming advanced development in autumn and enhanced survival in winter, but retarded growth and increased mortality in summer even at the northern limit of species's range. Our current studies are going to clarify that such an adverse effect of high temperature is caused by the decrease in bacterial symbiont which is harboured in the midgut of stink bugs.

Keywords:*range expansion, climate warming, southern green stink bug, interspecific copulation, extinction, diapause*

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S1014TH02

Integrated Pest Management

Pecky rice problem in Japan

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Two mirid bugs, Trigonotylus caelestialium and Stenotus rubrovittatus (Heteroptera: Miridae), are important pests that infest rice crops in Japan. In particular, the rice leaf bug, T. caelestialium, reproduces on gramineous plants and invades rice paddy fields after rice plant heading. Damage from the bug feeding causes stained grains, known as pecky rice. The females emit sex pheromone and attract conspecific males. Hexyl hexanoate, (E)-2-hexenyl hexanoate and octyl butyrate were identified as female sex pheromone components and the synthetic sex pheromone also attracts conspecific males. Sweeping of vegetation with an insect net is one of the conventional methods of surveying for insect pests in rice. Pheromone traps are easy to use and also eliminate the need for specialized training to detect and identify the target insects. A sticky trap with a rubber septum impregnated with the synthetic sex pheromone was placed in the center of a paddy field. The fluctuation pattern of males caught in traps and the number of males captured by sweeping were synchronized. This result suggests that the pheromone trap can be utilized as a monitoring tool for the rice bugs in paddy fields. Therefore, we have clarified the relation between the number of bugs caught in traps and the percentage of pecky rice, and developed the technique to determine the necessity and timing of chemical control.

Keywords:*mirid bug, rice leaf bug, pecky rice, pheromone trap*

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Research status on stink bugs in Korea

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Some species of true bugs have been gradually becoming serious problems worldwide on several crops. Some bugs are important as biological control agents of insect pests. Reviewing articles would be meaningful for the set-up of research direction for this insect group in the future. Thus, we reviewed the articles on true bugs studied in Korea since 1960. A total 122 papers on pest or natural enemy bugs have been published in scientific journals. Number of papers per decade is increasing, recording 82 papers since 2000. A nearly one third of total papers is related to the bean bug, Riptortus pedestris, mostly focused on its aggregation pheromone and natural enemies. This is perhaps because R. pedestris is an important pest of soybean, non-astringent (sweet) persimmon or sometimes of apple. Among cultivated crops, rice plant and soybean were most frequently dealt with by researchers. We categorized all the papers into several subjects; classification and morphology, development or growth analysis, bug fauna and damage in some crops, seasonal occurrence patterns, egg parasitoids of stink bugs and predatory bugs. Recently, research on the aggregation pheromone of R. pedestris has been actively done; the pheromone component composition of Korean population, attractiveness of the pheromone to itself and to other stink bug like Piezodorus hybneri and a parasitoid, Ooencyrtus nezarae (Encyrtidae). Attraction of the tachinid fly Gymnosoma rotundatim was reported to the pheromone of P. stali (Pentatomidae). Research on using the refrigerated R. pedestris eggs is actively going on to augment its parasitism in soybean field.

Keywords:*True bug, Sweet (non-astringent) persimmon, Soybean, Riptortus pedestris, Plautia stali, Parasitoids*

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Integrated Pest Management

Recent advances on phytophagous stink bug feeding and damage in South America

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Stink bugs (Heteroptera: Pentatomidae) are considered pests of economic importance of major commodities in the neotropics, such as soybean, wheat, corn, and cotton. They feed by introducing their mouthparts (stylets composed of a pair of mandibles and a pair of maxillae) on plant tissues, either vegetative, such as leaves and stems, or reproductive, such as seeds and fruits. Heteropterans use four types of feeding: stylet sheath, lacerate-and-flush, macerate-and-flush, and osmotic pump feeding. Before introducing the stylets, bugs probe the food with their mouthparts, and the information whether the food is suitable or not is apparently detected by gustatory sensila, present on the tip of the rostrum. After probing, stylet penetration occurs. The potential penetration of the stylets has been estimated based on the rostrum length, and on the probing position plus length of mouthparts segments. The resulting damage is caused by feeding time, mechanical and chemical stress. Mechanical stress is related to the mandible serration, that destroy plant tissue as they penetrate and move inside the tissue; chemical stress is due to the action of salivary enzymes that destroy the cell contents. Feeding damage to soybean seeds by four species of pentatomids [Dichelops melacanthus (Dallas), Euschistus heros (F.), Nezara viridula (L.), and Piezodorus guildinii (West.] will be presented and discussed in detail. Data showing wear of mandibles of stink bugs due to feeding activity on different food sources will be presented and discussed, as well.

Keywords:*Pentatomidae, stink bugs, seed damage, soybean, mandible serration, stylets penetration*

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S1014TH05

Integrated Pest Management

Current pest status of *Halyomorpha halys* in the USA

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Since its initial discovery in the late 1990's in Allentown, PA and subsequent identification in 2001, the brown marmorated stink bug (BMSB), Halyomorpha halys (Stal) has been detected in over thirty five states in the continental United States. In most states, BMSB is considered a nuisance pest. However, BMSB was shown to potentially impact tree fruit in Pennsylvania and New Jersey in 2006 and 2007. In 2010, BMSB populations exploded in the mid-Atlantic Unites States impacting a wide variety of agricultural crops throughout the mid-Atlantic area. The impact and ramifications of this population explosion will be discussed.

Keywords:*stink bugs, pest status, Halyomorpha halys*

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Egg parasitoids of stink bugs: Research status and potential for biological control

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Egg parasitoids are among the most important natural enemies associated with herbivorous stink bugs, and they show remarkable potential as biological control agents. On the other side, some egg parasitoid species also attack predaceous bugs, thus affecting the efficacy of these important entomophagous insects. Most studied stink bug egg parasitoids belong to Platygastridae (=Scelionidae) and Encyrtidae, the main research topics concerning behavior, chemical ecology, behavioural ecology and field efficacy. Some species have been used for biological control programs. However, some constraints may prevent egg parasitoids from becoming effective agents in inundative biocontrol projects, the most important being incomplete behavioral and ecological knowledge for most species and inefficient mass production. Thus, understanding the host selection strategies of egg parasitoids and the chemical stimuli involved would lead to enhanced biological control through behavioral manipulation of egg parasitoids in the field, and to the development of better rearing methods. Egg parasitoids of herbivorous stink bugs may adopt differing host selection strategies, the most important being the exploitation of kairomones from non-target instars in combination with host-induced plant synomones (indirect resistance), and associative learning. The chemically mediated behavior of these wasps may be exploited, e.g., through the development of plants with elevated expression of indirect induced resistance, or spraying fields with resistance elicitors. Important elements toward implementation include procedures for selection of egg parasitoids, evaluation of their specificity, risk assessment of new introductions, release methods, and quality control. Implementation of knowledge acquired from laboratory studies must finally be scaled up and transferred to growers.

Keywords:*Egg parasitoid, stink bug, biological control, ecology, behavior*

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Provision of host eggs to increase field parasitism of the bean bug: Theory and practice

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Andong National University, Republic of Korea

Augmentative biological control of Riptortus pedestris Thunberg (Heteroptera: Alydidae) using parasitoids is not economically feasible in field crops like soybeans in Korea due to the cost of rearing parasitoids and the relatively low cash value of soybeans. As a conservational biological control, the release of cold-stored eggs of R. pedestris has been suggested because the eggs become non-viable but can still be parasitized by parasitoids. This method has potential advantages: (1) it is cost effective and has no quality issues such as inbreeding depression and alteration of foraging behavior of parasitoids, and (2) it would minimize disturbance to the parasitoid community caused by the release of a certain parasitoid species. We evaluated inoculation of cold-stored eggs as a method to enhance field parasitism. First, about 10,000 cold-stored eggs were inoculated in soybean fields (2,028-3,540 m2) in 2006. A higher seasonal parasitism rate was found at the treated sites although pest populations were not affected. Second, because aggregation pheromone, produced by males of R. pedestris, also attracts egg parasitoids, we used cold-stored host eggs in the pheromone trap together to both trap bugs and propagate parasitoids on host eggs attached to the trap. About 10,000 non-viable eggs were used in soybean fields (1,677-2,844 m2) in 2008. Again, although significantly higher parasitism (78-91%) occurred in the treatment sites compared to control sites (0-62%), no statistical significance of treatment was found in R. pedestris population. Explanations for these outcomes and future studies needed to be done are discussed.

Keywords:*alydid, biological control, Ooencyrtus nezarae, Gryon japonicum, Riptortus pedestris*

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Detecting source habitat of the bean bug *Riptortus pedestris* and its egg parasitoid: Toward area-wide pest management using natural pest control

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Natural pest control is a major alternative to the chemical control of agricultural pests, but it needs improvement. Quantifying the abundance of insect pests and invertebrate natural enemies in source habitat would be essential for designing environmentally friendly crop-protection systems. To evaluate the availability of natural pest control for the soybean pest Riptortus pedestris (Hemiptera: Alydidae) by their major egg parasitoid Ooencyrtus nezarae (Hymenoptera: Encyrtidae) in source and arable habitats, we compared their abundance in soybean fields and at forest edges, which were assumed to be the overwintering site of R. pedestris in previous studies. We set traps baited with synthetic attractant for both species in soybean fields and along forest edges in mid-August over 2 years. During one of the two years, we also examined the degree of natural pest control by examining the rate of parasitism using an egg trap. The results showed that the numbers of both R. pedestris and O. nezarae trapped at forest edges were higher than those in soybean fields. Our result also showed that better pest control service by O. nezarae was provided at forest edges than in soybean fields. Therefore, when utilizing pest-control by O. nezarae in soybean fields, it is important to consider the spatial arrangement and distance to the forest edge. We would like to discuss about the importance of quantifying the abundance of insect pests and their natural enemies in source habitats beyond single-field boundaries when controlling insect pests using natural enemies.

Keywords:*landscape structure, conservation biological control, aggregation pheromone, Glycine max*

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Seasonal habitat use by stink bug pests and their egg parasitoids: Implications for managing these mobile pests in diversified agroecosystems

Yoshimi Hirose
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Stink bug pests are most notable among mobile pests defiend as polyphagous and multivoltine pests that move freely over large areas and between crops in diversified agroecosystems. Natural enemies of mobile pests have difficulty in shifting their habitats to follow the seasonal movements of the pests, and egg parasitoids are most important among natural enemies of stink bug pests. Thus, determining how stink bug pests and their egg parasitoids seasonally use their habitats is necessary for managing these mobile pests. Based on field surveys of egg parasitism of stink bug pests in Japan, I examined habitat use by these pests and their egg parasitoids during a growing season. Results of the surveys showed that some parasitoid species can shift their habitats to follow the seasonal host movement but others cannot. When a parasitoid species cannot shift its habitat to follow the seasonal host movement, its hosts escape parasitism in a new habitat. Proper host management is required for this habitat unless other parasitoid species reach the habitat. For managing stink bug pests, information on habitat use by their egg parasitoids during hibernation also is needed. Hibernation habitats of these egg parasitoids are different from their habitats during a growing season. Under the bark of zelkova trees in village shrines, I found overwintering aggregations of female adults of Trissolcus spp. which are egg parasitoids of some stink bug pests in Japan. On the basis of this finding, the importance of conserving the hibernation habitats of these egg parasitoids is emphasized.

Keywords:*Natural enemy, Habitat shift, Hibernation habitat, Conservation biological control*

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S1014TH10

Integrated Pest Management

Attractiveness of stink bugs to trap types and its utilization in fields

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Six species of stink bugs are regarded as major stink bugs on soybean, Korea. These are classified into two families of alydidae and pentatomidae taxonomically. Bean bug, Riptortus pedestris Fabricius, belonging to alydidae has high flying and moving ability while penatomidae stink bugs such as one-banded stink bug, Piezodorus hybneri Gmelin, brown-mamorated stink bug, Halyomorpha halys Mista, sloe bug, Dolycoris baccarum Linnaeus, green stink bug, Nezara antennata Scott and brown-winged stink bug, Plautia stali Scott have low flying and moving ability relatively. Several types of traps are generally used for attraction of stink bugs in agricultural fields. Fish-net trap and funnel trap are effective for attraction of R. pedestris while barrier-funnel trap and disk trap are effective for attraction of pentatomidae stink bugs generally. Recently, M-S cone trap which is developed by our own is also evaluated as an effective attraction of R. pedestris in soybean field. Accordingly, attractive effect of stink bugs is closely related with trap types. In addition to, attractiveness of stink bugs are significantly influenced with amount and composition of aggregation pheromone, trap placing height and site, and different lure combinations. In particular, attractive effect of stink bugs are significantly increased with aggregation pheromone-soybean seeds combination trap placed at plant height in fields. Accordingly, these factors are properly considered for the effective management of stink bugs population in agricultural fields by mass trapping with lures including aggregation pheromone.

Keywords:*stink bugs, trap, attraction, lures, utilization*

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The pentatomid hunters: Molecular tracking of predation on stink bugs (Hemiptera: Pentatomidae) in cotton and soybeans

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Stink bugs (Hemiptera: Pentatomidae) have emerged as significant pests of cotton and soybeans in the southeastern United States with losses exceeding $70 million annually. Three of the most prominent pests are the southern green, Nezara viridula, green, Chinavia hilaris and brown stink bug, Euschistus servus. However, their predators are poorly understood, especially in respect to early season predation. As field observation of predation is difficult, molecular gut content analysis offers an alternative to traditional approaches. Consequently, species-specific 16S molecular markers were designed and used to discern the gut contents of potential predators of stink bugs. Various predators were collected throughout the summer in cotton and soybean in southeastern Georgia, and predation frequency was examined relative to prey availability. Predation rates were significantly higher on N. viridula than on E. servus and C. hilaris, despite relatively similar availability of all prey resources. Our results showed that N. viridula was consumed primarily by the predators Coleomegilla maculata, Geocoris punctipes, and Notoxus monodon, with up to 40% positive in July. These data suggest generalist predators could provide valuable biological control of stink bugs, but additional research examining the spatio-temporal nature of predator-prey interactions is required to understand the structure of such food webs relative to pest management.

Keywords:*molecular ecology, predator-prey interactions, food web ecology, biological control*

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Bt maize in the Americas from 1996 to 2012

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Maize hybrids that are genetically engineered to produce crystal (Cry) proteins or toxins from the soil bacterium, Bacillus thuringiensis (Bt), have revolutionized pest control and currently are grown in more than a dozen countries on over 58 million acres. The primary targets of Bt maize are lepidopteran larvae that are stalk borers, ear or leaf feeders, and coleopteran larvae that feed on roots. Growers are attracted to Bt maize because of the reduced need for chemical insecticides, yield protection, improved grain quality, and convenience. Suppression of pest populations has benefited both Bt and non-Bt maize growers in the United States Corn Belt. Since the first commercial use of Bt maize in 1996 the variety of genetically-engineered maize products for insect control has increased substantially, which has expanded the spectrum of targeted pests and improved insect resistance management strategies. Yet in the Americas entomologists are learning that Bt maize must be used in concert with other insect management practices, a new approach to integrated pest management, because overreliance on a single tactic at least for some insect pests leads to insect resistance issues.

Keywords:*Bt corn, resistance management, integrated pest management*

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The changing landscape of cotton IPM in the GM crop era

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The adoption of GM cotton continues to expand with 12 countries producing nearly 25M hectares in 2011. The global economic and environmental benefits continue to accrue as well with an estimated reduction of >150M kg in insecticide active ingredient and farm income benefits totaling nearly $20B over the last 14 years for insect-resistant Bt cotton. The deployment of Bt cotton has affected regional reductions in target pest populations in several countries and these reductions have sometimes benefitted producers of other crops, especially with polyphagous target pest such as cotton bollworm. Regional reductions in target pest populations have also opened the door for more permanent solutions to pest problems. For example, Bt cotton has been a key technology in permitting the eradication of the longtime invasive pink bollworm from the USA and northern Mexico. Dramatic reductions in insecticide use aimed at Bt targeted pest has also been associated with significant increases in ecosystems services such as biological control. These affects have cascaded within agroecosystems, allowing more ecologically-based management of numerous key pests that are unaffected by Bt proteins. For example, Bt cotton has played a central role in reducing insecticide use in Arizona cotton by nearly 90% in the last 15 years. Despite these gains, reductions in insecticide use have also precipitated or enhanced problems with other pest species that were once controlled by the broad-spectrum treatments for Bt targeted pests. This presentation will highlight benefits and costs associated with Bt cotton production globally.

Keywords:*Bt Cotton,biological control, pink bollworm, landscape effects, non-target pests*

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Indirect, plant-mediated competition in Bt cotton - a possible mechanism underlying secondary pest outbreaks

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Genetically engineered cotton plants that express lepidopteran-active Cry proteins from Bacillus thuringiensis (Bt) are grown on millions of hectares worldwide and have lead to area-wide population suppression of key pest species. Numerous studies have established that the Cry proteins expressed in commercialized Bt cotton varieties are highly specific to herbivores belonging to the order Lepidoptera. A potential negative effect of the adoption of Bt cotton varieties is the emergence of secondary-pests and it has been reported from different world regions that populations of some non-lepidopteran species have increased. This increase in non-target herbivore populations has generally been linked to the reduced use of broad-spectrum insecticides to control pest Lepidoptera. Potential indirect, plant-mediated effects of Bt cotton on non-target herbivores, however, have received little attention. We have studied the impact of the insecticidal trait on the natural inducible defense mechanisms of cotton. Cotton is defended by a range of terpenoids (e.g., gossypol) that are induced by herbivore damage. We show that the reduction of damage caused by the elimination of caterpillars in Bt cotton can lead to a lower concentration of cotton terpenoids, thereby making the plant more vulnerable to attack by other herbivores such as aphids, which do not induce a defense response by the plant.

Keywords:*Aphis gossypii, Bt cotton, non-target pests, terpenoids, transgenic crops*

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S1015TH04

Integrated Pest Management

Bt rice in China: Potential benefits, impact, and sustainability

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From the ?rst insect-resistant genetically modi?ed (IRGM) rice transformation in 1989 in China. Numerous lines of genetically modified rice expressing cry genes from Bacillus thuringiensis (Bt) have been shown to confer resistance to lepidopterous pests under laboratory and field conditions. Until October 2009, the Chinese Ministry of Agriculture issued biosafety certi?cates for two transgenic cry1Ab/Ac rice ines, and China made a great leap forward from insect-resistant genetically modified (IRGM) rice basic research to potential commercialization of the world's ? rst IRGM rice. Research has been conducted on developing IRGM rice, assessing its environmental safety (mainly on non-targets herbivores, natural enemies, domestic silkworm and arthropod diversity as well as gene flow to wild rice) and food safety impacts, and evaluating its socioeconomic consequences. Laboratory and ?eld tests have con?rmed that these two Bt rice lines and other Bt rice lines can provide effective and economic control of the lepidopteran complex on rice with less risk to the environment than present practices. Commercializing these Bt plants, while developing other GM plants that address the broader complex of insects and other pests, will need to be done within a comprehensive integrated pest management program to ensure the food security of China and the world.

Keywords:*Bt rice, rask assessment*

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Bt transgenic grain legumes in pest management : Progress and problems

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Insecticidal genes from the bacterium, Bacillus thuringiensis have been inserted into grain legumes such as chickpea, pigeonpea, cowpea, faba bean, lentil, soybean, and groundnut. However, transgenic grain legumes have not yet been deployed for pest management. While efficacy of transgenic copwpea, chickpea and soybean groundnut has been proved under greenhouse conditions, only transgenic copwpea is being tested under field conditions in West Africa. To ensure a sustainable deployment of transgenic insect-resistant grain legumes, it is important that they are compatible with other control methods, including biological control agents that are of importance for natural regulation of pest populations. Toxin genes from Bt are considered to be environmentally benign and their use will reduce the hazards associated with the use of synthetic insecticides. However, it is important to follow bio-safety regulations, and there is a need for better presentation of the benefits of biotechnology to the general public for rational deployment of the transgenic plants for pest management and sustainable crop production. Incorporation of insecticidal genes in crop plants will have a tremendous effect on pest management. Emphasis should be placed on combining exotic genes with conventional host plant resistance, and also with traits conferring resistance to other insect pests and diseases of importance in a crop in the target region. There is a need to follow the biosafety regulations and make this technology available to farmers at a reasonable price, who cannot afford the high cost of seeds and chemical pesticides.

Keywords:*Grain legumes, transgenics, pest management*

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Bt vegetables for insect management within IPM programs: Successes, disappointments, challenges and ways to move forward

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Genetically engineered (GE) insect-resistant plants that express proteins from Bacillus thuringiensis (Bt) have been widely adopted for corn and cotton. However, the development and commercialization of Bt vegetables has lagged in comparison, which is unfortunate since vegetables tend to be insecticide-intensive crops due to high pest pressure and cosmetic standards required for the market. While it is often stated that consumer choice has played a major role in companies avoiding development of Bt vegetables, this concept requires re-evaluation. When consumers in North America have been provided basic information about Bt plants and given a choice between Bt and conventionally sprayed sweet corn, they have often preferred the former. Likewise, 77% of consumers in a US survey said they would likely purchase foods produced through biotechnology if it can reduce pesticide use. Presently, however, the only commercialized Bt vegetable is sweet corn. More critical obstacles to Bt vegetables are their relatively smaller acreages and the cost of government biosafety regulations that inadvertently favor large acreages of field crops because companies can obtain a better return on investment. In developing countries, private-public partnerships may provide a way to bring Bt vegetables to market. However, these can be subverted by misinformation from anti-biotech campaigns, as is the case with Bt eggplant in India. Without the use of Bt vegetables as a tool for integrated pest management, farmers and the general public will not be able to realize the substantial environmental and economic benefits that are well documented with Bt cotton and Bt corn.

Keywords:*GM crops, GE crops, Bt, Bacillus thuringiensis, insect resistant crops*

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Field resistance of Bt eggplant expressing insectidical Bt Cry 1Ac protein against the fruit and shoot borer, *Leucinodes orbonalis* Guenee in the Philippines

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The two seasons of multilocation field trials in Luzon Island, Philippines of open-pollinated transformed eggplants expressing the Cry 1Ac insecticidal protein from Bacillus thuringiensis subsp. kurstaki (Bt) were completed during the 2010-2011 cropping seasons. The field evaluations showed consistently high efficacy of the Bt eggplants against the field populations of fruit and shoot borer (FSB), Leucinodes orbonalis Guenee across seasons over three locations as compared to their nonBt counterpart. The parameters used to measure field resistance were shoot damage, fruits with holes and extent of larval tunnelling expressed as percentages. In addition, the effect of additional protection from chemical control with the use of Bt eggplant was also determined. The results showed that weekly insecticide application did not significantly reduced the high percent damage in nonBt eggplant while both treated and untreated Bt eggplant showed consistently reduced larval damage.

Keywords:*Host Plant Resistance, Bt eggplant, Eggplant Fruit and Shoot Borer, Leucinodes orbonalis*

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Insect-resistant GM crops and IPM: The chemistry should be right

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Integrated pest management (IPM) has shown excellent results in the development of durable management of insect pests. Yet, although successful IPM strategies have been developed worldwide, further improvement of IPM is ongoing and may be implemented in new crops. The use of transgenic technology to create insect-resistant crops can offer a solution to the limited availability of highly insect-resistant cultivars. On the other hand, the development of transgenic insect-resistant crops may provide a first step in the development of new IPM programmes to replace current control strategies that are based on chemical pest control. After the elimination of the primary pest through a GM approach, secondary pests need to be controlled through additional control methods, preferably methods that further support the development of an environmentally benign pest management strategy. This resembles the development of many IPM programmes. The GM approach and the IPM approach have usually been developed in separation rather than in collaboration. Yet, when the chemistry is right GM and IPM can prove to yield a fruitful integration that can lead to the enhancement of durable, environmentally benign methods of pest management.

Keywords:*biological control, host plant resistance, tritrophic interactions, IPM, predators, parasitoids*

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Insect resistance management for GE crops: Patterns of success

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University of Melbourne, Australia

After 15 years of field use, resistance to crops expressing insecticidal proteins modelled on those from Bacillus thuringiensis (Bt) has now evolved in at least 4 cases in the field. These include 3 Lepidoptera, the pink bollworm, Pectinophora gossypiella, to Bt cotton in western India, and to Bt maize, the armyworm, Spodoptera frugiperda, in Puerto Rico, and the stem borer, Busseola fusca, in South Africa. Among beetles, we have resistance to Bt maize in the chrysomelid Western corn rootworm, Diabrotica virgifera, in the USA. In other species and locations, increases in resistance allele frequencies have been observed. While the extent and losses to Bt resistance are still far less than to chemical insecticides used with similar intensity, increasing problems with resistance to Bt, and the lack of remedial strategies when resistance evolves, has highlighted the need to increase efforts to adopt tactics to delay resistance and use Bt more sustainably. As foreshadowed by models and experiments, the cases in which resistance has evolved clearly show the importance of significant and generally deliberately structured refuges, high expression of the Bt toxins relative to the susceptibility of the pests, and the use of pyramided varieties with multiple Bt genes each showing adequate expression. Successfully sustaining the use of Bt will likely require modest government involvement in requiring application of these resistance management tactics.

Keywords:*Bacillus thuringiensis, resistance management, Bt crops*

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Insect resistance management in GM crops: Challenges for African farmers

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North-West University, South Africa

The African maize stem borer, Busseola fusca (Lepidoptera: Noctuidae) developed resistance to Bt maize (Cry 1Ab) in South Africa, 7 years after release of Bt maize in the country. Since then resistance spread throughout the maize belt of the country where maize is produced in large scale commercial farming systems. The insect resistance management (IRM) strategy recommended for stem borers is the high-dose/refuge strategy in Bt maize and Bt cotton. For Bt maize, poor compliance to refuge requirements was blamed for resistance development in several cases in other parts of the world as well as South Africa where surveys showed that farmers only started to comply with requirements, 8 years after release of Bt maize. Non-compliance can however not be the only reason for rapid resistance development. Resistance development highlighted huge gaps in knowledge of pest biology (for example migration patterns) and interactions in the wider agro-ecosystem (indigenous host plants/parasitoids). IRM in Africa is faced by challenges unique to farming in subsistence and small scale systems where refuge requirements are unrealistic and difficult to manage and monitor. Furthermore, factors such as seed management practices, gene-flow to maize land races could be contributing factors to resistance development. Alternative IRM strategies such as seed mixtures are more practical but have its own challenges and have not been proven successful yet. While wild host plants of the African boll worm (Helicoverpa armigera) (Lepidoptera: Noctuidae) may contribute to unstructured refugia, this is not the case with any of the maize stem borers.

Keywords:*Bt crops*

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Controlling corn rootworm with dsRNA: Efficacy, specificity and IRM considerations

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The expression of dsRNA in crop plants, such as maize, to control damaging insect pests such as western corn rootworm (WCR, Diabrotica virgifera virgifera LeConte) represents a new class of insect control traits with a high degree of insect specificity. As with any new plant-expressed insect control trait, the potential for WCR to evolve resistance to dsRNA needs to be considered. This presentation will discuss information regarding pyramiding WCR-active dsRNA with Bt Cry proteins for increased efficacy and decreasing the potential for resistance development while maintaining a relative high degree of specificity in host spectrum

Keywords:*RNAi, dsRNA, transgenic crops, corn rootworm*

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Reconstructing insect invasion routes with genetic tools

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Insect invasion biology and agriculture are intimately related because many agricultural insect pests are recent invaders. In this paper we propose that reconstruction of invasion routes with population genetics-based methods can address fundamental questions in ecology and practical aspects of insect pest management. We briefly present the methods of invasion routes reconstruction and describe the main results recently obtained. In particular, we focus on a scenario ? the bridgehead invasion scenario ?, which had been overlooked until recently. We show that this scenario, in which an invasive population is the source of other invasive populations, is evolutionarily parsimonious and may have played a crucial role in insect invasion biology.

Keywords:*invasion pathways, Diabrotica virgifera, harmonia axyridis, WCR, asian ladybeetle*

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Population genetics and disease ecology of mosquito species in Galapagos

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The introduction of new pathogens and disease vectors has been recognized as a major threat to Galapagos Island biodiversity. Here I focus on mosquito species of the Galapagos archipelago, using population genetic and phylogenetic data to understand their historical and current population dynamics. I show that two mosquito species found in the archipelago have very different historical and contemporary evolutionary histories: one species, Aedes taeniorhynchus, naturally colonized the archipelago 200,000yrs ago and is now found widely in the islands, having adapted and spread to a range of different habitats. It has also changed its feeding-behaviour and now frequently feeds on reptiles in addition to mammals, unlike the continental progenitor populations. These properties potentially make Aedes taeniorhynchus a key bridge-vector in the archipelago for any new invading mosquito borne diseases. In contrast, I show that Culex quinquefasciatus, a major vector of diseases such as West Nile virus and avian malaria, has been introduced on multiple occasions since 1985 via human transportation networks and that its distribution and movement in the archipelago depend greatly on human activities. These two species might play an important role in the introduction and spread of new diseases in the Galapagos archipelago.

Keywords:*Population Genetics, Disease Ecology, Mosquito Species*

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Integrated Pest Management

Outbreeding depression and reluctant virgin oviposition in Californian populations of the bean thrips, *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae), reduce its invasive potential

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Bean thrips, Caliothrips fasciatus, is native to western North America. Once considered a pest of several crops in its native area, its pest status has waned over recent decades. However, due to its habit of aggregating in the navel of navel oranges, bean thrips remains economically important because some countries importing oranges from California have designated it a quarantine pest. That said, despite continued propagule pressure, bean thrips has never established outside North America. Using mitochondrial DNA we examined variation among Californian populations of C. fasciatus and found potential evidence of two cryptic species; a common widespread form (B), and a rarer form (A) with a very limited distribution. Form B showed strong phylogeographic structure and inter-population crossing experiments between three geographically isolated populations of form B revealed evidence of substantial outbreeding depression between these populations. A low frequency of offspring production by hetero-population pairs was evidence of pre-mating isolation, while post-mating isolation in this haplodiploid species was also evident in the elevated mortality of fertilized eggs in successful hetero-population crosses. One surprising finding was the total lack of offspring production by virgin females when isolated individually. However, virgin females did produce sons in the presence of other virgin females. These factors, acting alone or in concert, are likely to adversely affect the invasion potential of C. fasciatus and may explain why it has been unable to establish outside of its native range, from small founding populations accidentally exported in Californian navel oranges.

Keywords:*Thrips, population genetics, COI, quarantine pest, cryptic species*

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Invasion genetics of the western flower thrips in China: Evidence for genetic bottleneck, hybridization and bridgehead effect

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The western flower thrips, Frankliniella occidentalis (Pergande), is an invasive species and the most economically important pest within the insect order Thysanoptera. F. occidentalis, which is endemic to North America, was initially detected in Kunming in southwestern China in 2000 and since then it has rapidly invaded several other localities in China where it has greatly damaged greenhouse vegetables and ornamental crops. Using the mitochondrial COI gene and 10 microsatellites, eight of which were newly isolated and are highly polymorphic, we investigated the genetic structure and the routes of range expansion of 14 F. occidentalis populations in China. Both the mitochondrial and microsatellite data revealed that the genetic diversity of F. occidentalis of the Chinese populations is lower than that in its native range. Two previously reported cryptic species (or ecotypes) were found in the study. The divergence in the mitochondrial COI of two Chinese cryptic species (or ecotypes) was about 3.3% but they cannot be distinguished by nuclear markers. Hybridization might produce such substantial mitochondrial-nuclear discordance. Furthermore, we found low genetic differentiation (global FST = 0.043, P < 0.001) among all the populations and strong evidence for gene flow, especially from the three southwestern populations (Baoshan, Dali and Kunming) to the other Chinese populations. The directional gene flow was further supported by the higher genetic diversity of these three southwestern populations. Thus, quarantine and management of F. occidentalis should focus on preventing it from spreading from the putative source populations to other parts of China.

Keywords:*Frankliniella occidentalis (Pergande), the western flower thrips, population genetics, microsatellit*

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Putative source and social form of the red imported fire ant, *Solenopsis invicta* disclosed by molecular markers in China

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The red imported fire ant (RIFA), Solenopsis invicta, a damaging invasive pest, was first introduced to mainland China in 2005 in Guangdong province, spreading to Guangxi, Fujian and Hunan province thereafter. Six microsatellite loci were selected to detect genetic diversity among 238 samples from 6 geographical populations of RIFA. 45 alleles were tested in all the loci with the mean allelic number of 7.5 and the efficient allelic number of 18.75. Hardy-Weinberg equilibrium analysis revealed a remarkable genetic equilibrium and an obvious diversity at most of the loci in 6 geographical populations. The average polymorphism information content varied between 0.0000-0.8011. The average observed and expected heterozygosity ranged from 0.5740 to 0.7778 and from 0.6104 to 0.7068, respectively. FST value of different locus varied from 0.0595 to 0.1304, with the average value of 0.0961, indicating though high levels of intraspecific genetic variation existed, the interspecific genetic differentiation were moderate. Cluster analysis conducted by UPMGA separated 16 RIFA subpopulations in China into 4 categories, Guangxi, Yuexi and Fujian populations were firstly grouped, indicates they are might from the same source region. Zhusanjiao populations were separated into 2 categories, indicating another two separated source regions. Taiwan population became a separate unique group, indicating a low possibility of introduction to mainland China. Both monogyne and polygyne were presented in mainland China using Gp-9Bb alleles with multiplex PCR, but with polygynes dominant. The ratio of polygynous to monogynous colonies was 97: 23 in the 120 fire ant colonies collected, but differed as location varied.

Keywords:*Solenopsis invicta, microsatellite,source, social form, genetic diversity, Gp-9 alleles*

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S1016TH06

Integrated Pest Management

Distinct invasion pattern of an alien whitefly correlated with different dominant haplotypes

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It is poorly understood why particular populations of an alien species can establish and become invasive in an introduced region, whereas others do not. We show that different invasion patterns of an alien whitefly Bemisia tabaci biotype Q (also known as "Mediterranean species") in China and USA were associated with introduced cryptic haplotypes. Our long-term field survey conducted in Shandong, China and Arizona, USA in cotton and multiple other crops showed that B. tabaci biotype Q can displace well-established population of biotype B in China while biotype Q can only be detected in greenhouse ecosystems in the USA during the past several years. Based on molecular method and laboratory experiments, we revealed that the dominant haplotypes of biotype Q introduced in China and in USA differ in many biological traits, which may partially help explain the difference in its competition ability and thus in its invasiveness in introduced regions.

Keywords:*Biological invasion, Cryptic invasion, Bemisia tabaci, Invasion patterns*

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Using haplotypes to map fall armyworm (*Spodoptera frugiperda*) migration pathways in the United States

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Spodoptera frugiperda (J.E. Smith; Lepidoptera: Noctuidae) or fall armyworm is an important agricultural pest of a number of crops in the western hemisphere. Two morphologically identical host strains of fall armyworm exist, the rice-strain and corn-strain, with the latter inflicting substantial economic losses in corn in both North and South America. Fall armyworm does not survive severe winters so the infestations affecting North America originate from migrants that overwinter in Florida and Texas. A haplotype method was developed that could distinguish the corn-strain populations from these two locations and was used to delineate the migratory pathways. The results from five years of surveys will be described. We also examined the population movements of fall armyworm in the Caribbean and the magnitude of genetic interactions, if any, with populations from North, South, and Central America. The results indicate limited interactions between Puerto Rico fall armyworm and those from Brazil or Texas, but the potential for significant exchanges with populations in Florida. A better understanding of fall armyworm populations and their movement is critical for the development of strategies to predict infestation levels and eventually control this economically important pest in the United States.

Keywords:*fall armyworm, migration, Spodoptera frugiperda*

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Estimation of emigration from a forest insect outbreak using population genetic tools

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Population densities vary over time, and some species occasionally increase to very high numbers, for example during outbreaks. In such situations populations are expected to expand into new areas due to density-dependent dispersal and sometimes even result in range expansion. We investigated if an outbreak in a local population of the northern pine processionary moth Thaumetopoea pinivora had resulted in establishment of populations in suitable habitats outside of the outbreak area. Six small populations were found that could potentially have originated from the outbreak area. Using data from 12 microsatellite markers and a variety of population genetic analyses we could conclude that these populations did not originate from the recent outbreak. Genetic variability was not reduced in these small, isolated populations and there were several unique alleles, indicating instead a different population history. In addition, there was apparent genetic isolation by geographic distance, implying that despite the high density of the outbreak population, significant gene flow has not occurred.

Keywords:*outbreak, expansion, forest pest, population genetic tools*

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On the potential of population genetics to refine scales for locust management

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Linking demographic and genetic dispersal measures is of fundamental importance for movement ecology and evolution. However, such integration can be difficult, particularly for highly fecund species that are often the target of management decisions guided by an understanding of population movement. Here, we present an example of how the influence of large population sizes can preclude genetic approaches from assessing demographic population structuring, even at a continental scale. The Australian plague locust, Chortoicetes terminifera, is a significant pest, with populations on the eastern and western sides of Australia having been monitored and managed independently to date. We used microsatellites to assess genetic variation in 12 C. terminifera population samples separated by up to 3000 km. Traditional summary statistics indicated high levels of genetic diversity and a surprising lack of population structure across the entire range. An approximate Bayesian computation treatment indicated that levels of genetic diversity in C. terminifera corresponded to effective population sizes conservatively composed of tens of thousands to several million individuals. We used these estimates and computer simulations to estimate the minimum rate of dispersal that could account for the observed range-wide genetic homogeneity. The rate of dispersal between both sides of the Australian continent could be several orders of

Keywords:*approximate Bayesian computation, migration rate, population size, power test, management unit, Orthoptera*

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Understanding dispersal and movement of *Aphis glycines* to improve durability of aphid resistant soybean

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The soybean aphid, Aphis glycines, has become the most important soybean insect pest in the North Central US region since its first discovery in 2000. By screening thousands of soybean varieties, several have been discovered with host-plant resistance to the soybean aphid. Currently there are 4 formally described resistance genes (termed Rag for Resistance to Aphis glycines), with Rag1 being commercially released in 2010. However, soybean aphid populations have been found that can overcome Rag-based resistance, despite the lack of intense selection pressure. Aphids that have overcome resistance offered by Rag plants threatens the efficacy and sustainability of host-plant resistance as an aphid management tactic. Therefore, research is needed to describe both the genetic mechanisms for soybean aphid adaptation and the potential for this adaptive genetic variation to spread across the soybean growing region. Data will be presented on progress towards identifying the genetic mechanisms, evaluating signatures of selection on these traits in natural populations, molecular profiling of soybean aphids collected on different Rag soybeans, and defining the influence of host alternation on population genetic structure. Implementation of this data towards the development of strategies that delay the spread of adaptation and extend the durability of host plant resistance will also be discussed.

Keywords:*Aphis glycines, aphid genetics, host-plant resistance, population genetics*

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Genome scans for adaptation to transgenic Bt maize

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Genome scanning involves surveying large numbers of markers in populations subject to diversifying selection. The goal is to identify the small minority of markers at which allele frequencies are diverging between populations more rapidly than would be expected as a result of genetic drift. These markers may then be considered as potentially located in regions of the genome where selection is acting. Although genome scanning has proven problematic when studying natural populations, it has considerable promise as a tool for studying laboratory populations that have been subjected to selection regimes. Here we consider the case of laboratory populations of the western corn rootworm, Diabrotica virgifera virgifera that have been maintained on transgenic maize varieties expressing insecticidal Bt toxins versus populations maintained on non-transgenic isolines. A simulation approach is used to investigate the density of genetic markers required to achieve reasonable power to detect selection.

Keywords:*selection, population genetics, Diabrotica, maize*

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S1016TH12

Integrated Pest Management

Paratransgenic symbionts that express targeted toxins in the termite gut

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Formosan Subterranean Termites (FSTs). FST workers harbor a complex microbial community of protozoa and bacteria in their hindguts on which they depend for survival. Therefore, this microbial community can be exploited as a novel, pesticide-free tool for termite control. We have shown that antimicrobial peptides (e.g., Hecate) kill the protozoa in the-termite gut. Termites starve after the loss of their gut protozoa, since protozoa are necessary for cellulose digestion. The addition of a ligand, which binds the antimicrobial peptide to the protozoa, increased the specificity and efficiency of the antimicrobial peptide against termite gut protozoa. In this study, we engineered termite gut bacteria as "Trojan Horses" to express antimicrobial peptides in the termite gut and spread them among colony members to eliminate the colony. To achieve this goal we (1) cultured bacteria from the FST hindgut and identified them using 16S rRNA gene sequencing, (2) selected bacteria with high tolerance to Hecate and/or ligand-Hecate in bioassays, (3) engineered bacteria to express Hecate or ligand-Hecate via plasmid transformation and/or integration into the chromosome using transposons, and (4) tested the engineered bacteria for their ability to kill protozoa in vitro (protozoa culture) and in vivo (feeding to termites, termite mortality assays).

Keywords:*Gut bacteria, protozoa, genetic engineering, Trojan Horse*

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Gene flow and neighborhood area in European corn borer (Lepidoptera: Crambidae) in the U.S., and implications for insect resistance management

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The European corn borer (Ostrinia nubilalis) is a major invasive pest of corn in the US and is the main target of transgenic Bt-corn expressing Cry1Ab or Cry1F toxins. Because of the high adoption of Bt-corn, there is intense selection pressure for resistance, and farmers must plant a nonBt refuge as part of an insect resistance management (IRM) plan. Effectiveness of IRM depends on gene flow among corn borer populations. We have used population genetics approaches to estimate gene flow, but interpretation of results has been complicated by the history of invasion which can affect genetic signals. Interpretaions are also complicated in North America by the presence of partially reproductively isolated races with different pheromone systems. Many areas in the northeastern U.S. are characterized by high topographic relief, which may serve to isolate some populations through barriers to dispersal, affecting gene flow and the potential for resistance evolution. We report preliminary gene flow estimates based on microsatellite and SNP markers, both within and between pheromone races in areas where topography may affect dispersal. We found that topography may play a role in limiting dispersal in the E pheromone race, but not in the more common Z race. Although gene flow in the E race is high, it is more restricted than in the Z race. We used an independent demographic estimate of population density to calculate Wright's neighborhood area. The results suggest that >13% of moths disperse >12 km/generation before ovipositing. Overall, our results indicate effective dispersal over long distances.

Keywords:*Ostrinia nubilalis, gene flow, dispersal, population genetics, insect resistance management*

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Genetic differentiation and gene flow among different geographical populations of *Ostrinia furnacalis* in China based on multiple molecular markers

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Investigations on genetic differentiation and gene flow among geographic populations of the Ostrinia furnacalis from China were detected by using both microsatellite and mitochondrial DNA markers.The results showed high genetic diversity in mtDNA genes of O. furnacalis populations. Practical microsatellite system for O. furnacalis population studies was developed based on microsatellite loci reported in O. nubilalis. Six polymorphic SSR loci were estimated by GeneScan sequencing technology. Two loci showed deviation from Hardy-Weinberg equilibrium (P-value < 0.05). The genotypic data showed no significant linkage disequilibrium between pairs of loci (P > 0.05). Topological structure of the phylogenic tree demonstrated no geographic clustering was observed. There was low correlation between Fst / (1 - Fst) and geographical distance (r = 0.135, P = 0.0025). Analysis of molecular variance results revealed that most of the genetic variation was within populations; less variation was detected among populations within each corn region, which suggested no obvious genetic differentiation among these corn regions. The degree of genetic divergence and gene flow varied within different corn regions. The relatively lowest Fst and highest degree of gene flow (Nm) was among populations in Huanghuaihai Summer Corn Region, while there detected significant genetic differentiation and relatively low Nm among populations in both North Spring Corn Region and Southeast Hilly Corn Region. In addition, results of Tajima's D and Fu's Fs neutrality tests implied that there might not be a population expansion in recent time and the Asian corn borer populations was at demographic equilibrium.

Keywords:*Ostrinia furnacalis, geographic population, genetic structure, genetic differentiation, gene flow.*

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Pattern of dispersal and gene flow of cotton boll weevil in North America

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The boll weevil (Anthonomus grandis Boheman) is an invasive insect pest of cotton that underwent a well documented range expansion across the southeastern U.S. from Mexico beginning about 120 years ago. The magnitude and pattern of genetic differentiation among boll weevil populations from North America across eight U.S. states and northern Mexico were evaluated using microsatellite loci analysis. Measures of genetic diversity (allelic diversity and heterozygosity) were greater in Southern than Northern populations, and were greater in the west than the east among Northern US populations. Classical F-statistics and Bayesian-based clustering analysis suggested that boll weevil populations were genetically structured across the geographic range sampled. South-Central US populations exhibit classic isolation by distance, indicating genetically stable populations, but evidence suggests that populations within the Eastern and Western regions have not yet reached genetic equilibrium. Gene flow appears to be relatively high among populations within the Eastern region. Population assignment data and estimates of gene flow indicate that migration between locations separated by several hundreds of km is frequent. Several case studies for North American boll weevil populations imply that the database of microsatellite genotypes makes it possible, through population assignment techniques, to identify the most likely geographic source of a boll weevil reintroduced to an eradication zone, which helps action agencies decide the most appropriate mitigation response.

Keywords:*boll weevil, dispersal, eradication, gene flow, genetic variation, invasive species, microsatellites*

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Regional and global population structure of the oriental fruit moth, *Grapholita molesta*

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Invasive pest species have significant impacts on agricultural crop yields. Understanding the population dynamics of invasive species is key to their management, and hence to ensure food security in the face of the increasing pressure on agricultural productivity imposed by the twin challenges of human population growth and climate change. The oriental fruit moth, Grapholita molesta is an economically destructive pest of stone and pome fruit, including peach and apple. From its putative native range in Asia, G. molesta has spread throughout the temperate regions of the world over the past century, and is further expanding its distribution range polewards. We used polymorphic microsatellite markers to examine 1) population genetic structure of G. molesta in a fruit-growing area in Northern Italy, and 2) population genetic structure of G. molesta worldwide. At the regional scale, population structure of G. molesta was relatively low, and could be explained by both active dispersal and passive dispersal via anthropogenic movement of fruit, bins and nursery material. At the global scale, population structure of G. molesta generally reflected continental divisions. There was little evidence for multiple introductions to each continent, or for admixture between populations from different origins, suggesting that area-wide spread via transport of fruits and other materials is a major mechanism of ongoing invasion. Consistent evidence that the spread of G. molesta is aided by trade-related human activities urge for improved community-based control measures, and for more stringent regulations regarding the transport of plant material.

Keywords:*oriental fruit moth, invasive pests, microsatellites, human-mediated dispersal, genetic structure*

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The Molecular Phylogeny and population genetics of the rice grasshopper, *Oxya* genus

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The grasshoppers of the genus Oxya are well known to damage rice. We investigated the phylogenetic relationship of Oxya genus from 91 individuals of nine Oxya species by using mitochondrial cytochrome b gene sequence analysis. The results showed that the nine Oxya species formed four well-supported clades. Seven populations were sampled to investigate the genetic differentiation among grasshopper populations with different hosts (i.e. wild vs. cultivated rice). We found significant genetic differentiation between groups collected from different hosts. The results suggested that the anthropogenic activity in cultivated rice fields could have played an important role in shaping the genetic structure of O. japonica. 100 individuals of Oxya japonica were analyzed by using COI DNA sequences collected from four Southeast Asian countries. The results showed that high genetic variance among groups were found which indicating a relatively strong geographic structure. All clades diverged from a common ancestor, and deep divergences were happened related to specific geologic events. The expansion times of southwest China and north Philippines groups were 92820 years ago and 70940 years ago, respectively. Oxya intricata of 12 populations were collected from four Southeast Asian countries by using mitochondrial DNA COI sequences and AFLP. Phylogenetic analysis and TCS indicated three deep monophyletic clades that approximately corresponded to three geographical regions separated by high mountains and a deep strait. In conclusion, the past geological events and climatic fluctuations were important factors on the phylogeographical structure and genetic patterns of O. intricata in Southeast Asia.

Keywords:*Oxya, Molecular phylogeny, Population genetics, Phylogeography*

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S1017TH01

Integrated Pest Management

Planthoppers threatening rice in Asia again? Why?

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In the last 5 years rice planthoppers (Nilaparvata lugens and Sogatella furcifera) have become the most damaging pests of rice. From 2008 Thailand's rice bowl has suffered continuous outbreaks for 9 consecutive seasons. In 2010 rice production lost 1.1 million tons of paddy and this year another loss of about 1 million tons is expected. Similarly Indonesia is suffering the same threats and had lost about a million tons in 2011. Smaller patches of outbreaks occur in India, Myanmar, Bangladesh, Philippines and India while China continues to lose about 1 million tons a year. Besides economic loss, hundreds of farmers suffer crop failures, falling in debts and poverty, pesticide poisoning and even suicides. Planthoppers are induced secondary pests that normally under natural control. Outbreaks are symptoms of unsustainable practices that destroy vital ecosystem services triggering exponential growth. Although abnormal weather like droughts and floods can trigger outbreaks, the most consistent factor in Asia seems to be insecticide misuse. The presentation describes the economic and social impacts of the outbreaks and looks at 3 ecological principles and the management strategies that might have contributed. It will discuss the need to take a broader view to consider social, marketing, structural and policy issues beyond the development technological fixes in order to prevent future outbreaks.

Keywords:*Planthopper outbreaks, ecosystem services, insecticide misuse, pesticide marketing*

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Occurrence and migration of *Nilaparvata lugens* in the Vietnamese Mekong Delta

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The brown planthopper, Nilaparvata lugens, is one of the most important insect pests of rice in the Vietnamese Mekong Delta. Virus diseases transmitted by the insect, for example, severely spread in the delta in 2006 and the government stopped rice exporting for their food security. The level of insecticide resistance of the N. lugens populations in this region is much higher than that of populations in the northern Vietnam. Therefore, their migration in the delta is a chief concern, but not fully understood so far. To access a whole image of occurrence and migration of N. lugens in the delta, a new technique was employed by using one material and two methods: a dense light trap monitoring data, analytical image of paddy fields from the MODIS satellite and trajectory analysis to suggest the insects' movements. The monitoring data in each province indicated clear seasonal and generational occurrence patterns. Generational occurrence peaks with an interval of about 30 days indicated that the population consisted mainly of insects locally multiplied. Additionally, the peaks of the paddy area at harvesting time estimated by the remote sensing corresponded well to that of the insect occurrence. This fact suggested N. lugens's dispersion and migration occurred in synchronization with rice harvesting. Finally, the distance of N. lugens migration in the delta was estimated by the new technique and found to be about 100 km. The distance is less than one-tenth of that of the long-migratory East Asian population.

Keywords:*Nilaparvata lugens, migration, remote sensing, rice phenology*

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S1017TH03

Integrated Pest Management

The return migration of rice planthoppers: Gaps in the loop

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The rice planthoppers (RPH) migrate regularly northward in spring and summer and southward in autumn, that perform a perfect loop between Indo-China Peninsula and the mainland of China. However, some gaps in the loop were noticed during a field survey in northern Viet Nam in November of 2009. Rice is the only food for RPH, but there was no rice planting in mid and north Viet Nam during a 3-months interval between the two cropping seasons (summer-autumn rice and winter-spring rice). Although the ratoon rice and the seedlings of the falling grain may provide foods and habitats for the returned hoppers, but very few RPH could be seen on them during our field surveys in 2011 and 2012. Also, simulating the flying pathways of the emigrants illustrated that it would be impossible for the RPH taking off from Mekong delta and other southern provinces to enter into mid and north Viet Nam. Even for the emigrants from Quang Nam and Quang Ngai, there would be also very few opportunity (< 20%) to arrive in mid Viet Nam. So the problems arise. How will the returned RPH populations survive with no food in so large area and so long time? How do the source populations of RPH rebuild in the winter-spring rice fields? Especially, how dose the southern rice black-streaked dwarf virus complete its infection cycles? And, is the pied piper playing his magic pipe? More detailed studies will be needed to reveal the mystery of the returned hoppers.

Keywords:*Nilaparvata Lugens, Sogatella furcifera, return migration, pied piper effect*

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How are *Nilaparvata lugens* in East Asia adapted for long-distance migration: Differences in a few migration-related characters between the planthoppers in the tropics and temperate regions?

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Accumulated information for the last 40 years has revealed that Nilaparvata lugens in East Asia make annual long-distance migration with assist of the wind system: northward movement in early summer and probable return migration in autumn. We compared few migration-related characters of N. lugens collected at various locations in East Asia and tropical Southeast Asia to identify their possible adaptation for migration. Macropters in East Asia had longer pre-ovipositional periods (7.6 days) compared to tropical macropters (4.7 days), which is presumably beneficial for pre-reproductive migration. Macropters maximized their feeding at the first few days after adult moult. This post-eclosion feeding markedly increased starvation tolerance in East Asia macropters relative to tropical ones: the tolerance of macropters after 76 hr. feeding increased 2.5 and 1.6 fold compared to unfed ones, respectively. Thus, East Asian macropters are 1.6 fold more resistance to starvation than tropical ones with the same conditions after feeding. These findings suggest a difference in the allocation of resources between East Asian and tropical N. lugens. In East Asian macropters, feeding was maximized at the first 2 days and this energy intake was invested in reserves. Feeding reduction on day 3 approximately coincide with maximum starvation tolerance, suggesting the timing of take-off from paddies. On the other hand, feeding by tropical macropters was maximized in the first 3 days. Much of this energy intake was probably invested in vitellogenesis. I further discuss how East Asia N. lugens has evolved in spite of the great disadvantage of prolonged oviposition.

Keywords:*Nilaparvata lugens, Brown planthopper, Migration, Oviposition, Starvation tolerance, Post-eclosion feeding*

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S1017TH05

Integrated Pest Management

Genetic background of the migratory capability of the brown planthopper, *Nilaparvata lugens*

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We generated four pure lines of the brown planhopper, Nilaparvata lugens, each showing one predominant wing form and body color, through the successive selection of emergent adults with these characteristics from a group of specimens collected in the fields and reared at high density: blackish short-winged (brachypterous) (BS), blackish long-winged (macropterous) (BL), yellowish short-winged (YS) and yellowish long-winged (YL) Repeating a series of crossing experiments in every possible combination with these four pure lines these 20 years, we propose here a two-major gene model for wing form regulation: one gene locus on an autosome and another locus on X sex chromosome, assuming short-wing inducing gene and long-wing inducing gene as allele, each additively functioning to induce the target wing form. We also show that a similar two-major gene model for body color regulation, assuming blackish body color inducing gene and yellowish body color inducing gene as allele. These models explaine well all of the results obtained by crossing experiments. The four lines differ also in several life-histry traits: the the developmental time is in the order of BS < BL= YS < YL; pre-obiposion period, in the order BS < YS < BL = YL, and also in egg period, longevity and fecundity, suggesting that the genes related to regulation of wing form and body color expression are also involved in the regulation of life histry traits. The pleiotrophic effects of these genes would adapt the hoppers to survive in various circumstances which they would encounter after migration.

Keywords:*wing polymorphism body color genetic variation life specific trait*

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Regional surveillance of rice plantoppers in Asian rice area

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The regional rice planthopper surveillance network had been established since April 2011 with the participation of Vietnam (South and North), Cambodia, Thailand, Philippines, Indonesia, Bangladesh, Laos, and Nepal. Through the surveillance network, the rice planthoppers and transmitted virus diseases can be intensively monitored then shared the information along the participated region. The surveillance activities include daily light trap catch and weekly yellow sticky trap sampling in the monitoring rice fields. This activities are the first internationally organized attempts to monitor actual field population, which have normally been ignored. The field monitoring data can be used to facilitate the prediction of adults emigration and to understand how each of the rice region connected to the others in terms of the rice planthopper occurrence.

Keywords:*rice, planthopper, surveillance, prediction, migration*

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S1017TH07

Integrated Pest Management

On-farm management of rice varieties for increased efficiency in planthopper population regulation

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International Rice Research Institute, Philippines

Planthoppers have reemerged as a considerable threat to rice production in irrigated rice-growing regions throughout Asia. Evidence suggests that planthopper populations are sensitive to perturbations of predator and parasitoid densities and efficiencies. The most popular approaches for reducing planthopper densities have included planting of resistant varieties and the use of chemical insecticides; however, evidence suggests that the efficiency and durability of resistant varieties are depleted under the influence of insecticides. Furthermore, the use of high levels of nitrogen fertilizers has been shown to reduce resistance in some plants. This talk examine the interactions between host-plant resistance, chemical fertilizers, and insecticides in determining the efficiency of planthopper population increase and regulation in rice paddies. It proposes a system of resistance management for increased durability of non-renewable resistance genes.

Keywords:*Brown planthopper, white-backed planthopper, resistance, insecticides, fertilizer, population regula*

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Plant penetration behavior of the rice sap-sucking Homopterans on susceptible and resistant rice varieties using DC-EPG system

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The stylet penetration behavior of four rice sap-sucking homopteran pests, Nilaparvata lugens, Sogatella fruciferra, Laodelphax striatellus, and Nephotettix cincticeps in rice plants (Oryza sativa) was evaluated by using an electrical penetration graph (EPG). We classified the EPG waveforms of planthopper group into seven different patterns, np, N1, N2, N3, N4-a, N4-b, and N5, according to their shapes, voltage amplitudes, voltage levels, and frequencies. The N4-b pattern was always preceded by N3 and N4-a, in that order. Continuous honeydew excretion only occurred during the N4-b period, and the plant sap flowed out of the severed stylets in the N4-b period consisted of sucrose mainly. On the other hand, we classified the EPG waveforms of the green rice leafhopper, N. cincticeps into seven different patterns, Nc1, Nc2, Nc3, Nc4, Nc5, Nc6, and Nc7. The Nc6 pattern was always preceded by the Nc5 pattern. Honeydew was periodically excreted only in the Nc6 pattern. The N4-b pattern of the planthopper group and the Nc6 pattern of the leafhopper were carefully considered as a phloem sap feeding behavior. However, the planthopper group and the leafhopper hardly showed the phloem sap feeding pattern on resistant rice varieties during an EPG-recording. In addition, the duration of the phloem sap feeding patterns was highly decreased on resistant rice varieties relative to susceptible ones. These results suggest that the phloem sap feeding patterns (N4-b and NC6) are an important parameter to determine resistance of rice plant against rice sap-sucking homopteran pests.

Keywords:*host plant resistance, EPG, plant penetration behavior, rice planthoppers, green rice leafhopper*

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Rice volatile signals are important regulators of rice-brown planthopper interactions

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In response to insect attack, plants release complex blends of volatile compounds. These volatiles serve as foraging cues for herbivores, predators and parasitoids, leading to plant-mediated interactions within and between trophic levels. Hence, plant volatiles may be important determinants of insect community composition. To test this we created rice lines that are impaired in the emission of two major signals, S-linalool and (E)-β-caryophyllene. We found that inducible S-linalool attracted predators and parasitoids as well as chewing herbivores, but repelled the rice brown planthopper Nilaparvata lugens, a major pest. The constitutively produced (E)-β-caryophyllene on the other hand attracted both parasitoids and planthoppers, resulting in an increased herbivore load. Thus, silencing either signal resulted in specific insect assemblages, a finding that highlights the importance of plant volatiles in determining population density of the rice brown planthopper and insect community structures. Moreover, the results imply that the manipulation of volatile emissions in crops has great potential for the control pest populations.

Keywords:*rice, plant volatile signal, tritrophic interaction, Nilaparvata lugens, plant-insect interaction*

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Exploring the factors underlying variation in brown planthopper-rice interactions

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Populations of the brown planthopper, Nilaparvata lugens, appear to vary both spatially and temporally in their performance on resistant rice varieties. Given that breeding for host plant resistance has been a central focus for planthopper management in rice, it is critically important to understand the factors that appear to underlie geographic variation in planthopper populations, and how they may change over time in response to resistant rice varieties. Here, we present two datasets: 1) The first examines population genetic structure of N. lugens throughout Asia, and 2) the second is a full factorial study examining how the reared host plant, exposed host plant, number of generations, and yeast-like endosymbionts may influence planthopper nymphal development and amino acid composition. We found a high level of population genetic structure throughout Asia, suggesting that planthoppers tend to exhibit a high level of inbreeding. Nymphal development was strongly influenced by a four-way interaction between the exposed rice variety, natal rice variety, number of reared generations, and YLS presence. Aposymbiotic planthoppers reared for more generations had a higher proportional concentration of rare amino acids than those reared for 8 generations, indicating that the selected planthopper populations appeared to improve their ability to acquire rare amino acids. Therefore, the change in amino acid composition of planthoppers suggests an underlying change in protein expression or amino acid metabolism over time.

Keywords:*Nilaparvata lugens, population genetics, host plant resistance, amino acid composition, rice*

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Recent situation of rice viruses transmitted by planthoppers in Southeast Asia

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Rice viruses such rice grassy stunt virus (RGSV), rice ragged stunt virus (RRSV), and Southern rice black-streaked dwarf virus (SRBSDV) transmitted by planthoppers gave serious damages to rice production in Southeast Asian countries. These viruses can independently infect rice plants. However, incidences of mixed infection of rice with RGSV and RRSV both transmitted by brown planthoppers (BPH) were often observed in countries like Vietnam and Indonesia. Also, mixed-infection of rice with SRBSDV, which is transmitted by white-backed planthoppers, and RRSV was also frequently observed in Northern and central parts of Vietnam. In fields, in addition to cultivated rice plants, weeds such as Echinochloa crus-galli and wild rice species such as Oryza rufipogon were found to serve as a reservoir for RGSV and RRSV. In Southern Vietnam, overlaps among cropping seasons across the rice cultivation areas, and overlaps between the BPH migration patterns and rice cropping patterns might have been the major cause of the outbreaks. In addition, predominant cultivation of rice varieties which are susceptible to BPH and the viruses might have facilitated the outbreaks. Examination of transmission characteristics of the viruses by BPH indicated that the transmission can be completed in a very short time (less than 20 min) in Southern Vietnam. These facts should be considered to establish a more effective management strategy for rice viruses transmitted by planthoppers in Southeast Asian countries.

Keywords:*Rice viruses, BPH, WBPH*

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Imidacloprid resistance in the brown planthopper: Different stories in lab and field

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Neonicotinoid insecticides are selective agonists of insect nicotinic acetylcholine receptors (nAChRs) and are used extensively to control a variety of insect pest species. The brown planthopper (Nilaparvata lugens), an insect pest of rice crops throughout Asia, is an important target species for control with neonicotinoid insecticides such as imidacloprid. Previous studies identified a point mutation (Y151S) in two different nAChR subunits (Nlα1 and Nlα3) from a selected lab strain of N. lugens with high resistance to imidacloprid. In heterologous expression system, such mutation had been confirmed to contribute to imidacloprid resistance in N. lugens as the target insensitivity. Interestingly, although studies are now underway to examine the prevalence of the Y151S mutation in field populations, to date there is no evidence to indicate that this laboratory-selected target-site mutation is responsible for resistance in field populations of N. lugens. When screening the changes in detoxification enzymes in field populations with different levels of imidacloprid resistance (ratio=17-82 fold), a P450 monooxygenase gene, CYP6AY1, was found high expressed in all field populations, with 4.3-9.0 fold increase in mRNA levels. By expressing in E. coli cells, CYP6AY1 was found to metabolize imidacloprid with high activity. When CYP6AY1 mRNA levels were reduced by RNA interference, imidacloprid resistance was lowered. In conclusion, the biochemical mechanism based on P450 monooxygenase activity increase might be the main mechanism for imidacloprid resistance in field populations, when the target site mutation is not prevalent at present.

Keywords:*Nilaparvata lugens, imidacloprid, point mutation, P450 monooxygenase*

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S1017TH13

Integrated Pest Management

Species and area specific development of insecticide resistance in Asian rice planthoppers

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Outbreaks of the brown planthopper (BPH), Nilaparvata lugens (Stal) and the whitebacked planthopper (WBPH), Sogatella furcifera (Horvath) have occurred in East Asia and Indochina since 2005. Outbreaks of the small brown planthopper (SBPH), Laodelphax striatellus (Fallen) has also occurred in China and Japan since mid-2000s. These outbreaks are closely related to the development of insecticide resistance of the populations in these regions. The insecticide susceptibility of the three planthoppers in Asian countries was determined and compared from 2006-2010 by topical application method. A species-specific change in the insecticide susceptibility was found in BPH and WBPH: imidacloprid resistance in BPH and fipronil resistance in WBPH. Resistance to imidacloprid has developed only in BPH in East Asia (Japan, China and Taiwan) and Vietnam, but not in the Philippines. In contrast, resistance to fipronil has developed only in WBPH in East Asia, Vietnam, and the Philippines. The LD50 values for imidacloprid in BPH in East Asia and Vietnam increased from 2006 to 2009, and have slightly decreased in 2010. In SBPH, an area-specific insecticide resistance has developed in Eastern China and Western Japan: the SBPH populations in Jiangsu Province, China showed resistance only against imidacloprid, whereas the populations in western Japan showed resistance only against fipronil. Migration of SBPH from Eastern China to Western Japan occurred in 2008; the following year local SBPH populations in Japan became resistant to both imidacloprid and fipronil. These populations were conceivably produced by the intercrossing between immigrant and domestic populations.

Keywords:*insecticide resistance, imidacloiprid, fipronil, topical application, migration*

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Integrated Pest Management

Mechanisms of pesticide-induced resurgence of the brown planthopper (BPH) *Nilaparvata lugens*

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Stimulation of reproduction involved in direct stimulatory effect of pesticides on fecundity of BPH females and indirect stimulatory effect via physiological and biochemical alterations of rice plants due to pesticide application. These changes are beneficial to feeding, survival and fecundity of BPH. Studies of physiological and molecular mechanisms of pesticide-induced stimulation of fecundity showed that pesticides activated RNA transcript level in fat body and resulted in significant elevation of the gene expression level of vitellogenin (Nlvg) in fat body, which is regulated by the juvenile hormone esterase (Nljhe), which pesticides suppressed the expression of Nljhe and resulted in a significant elevation of JH level. Insecticides also stimulate the reproduction of adult males. The content of accessory gland proteins (AGPs) of treated males significantly increased. This stimulatory effect could be transferred to the reproduction of adult females via mating. Mating pair combinations of treated males and untreated males with treated females or untreated females showed that the number of eggs laid by the mating of treated males and treated females was significantly higher than that that of untreated males with treated females, showing that insecticide-induced the stimulatory effect of males could be transferred to the fecundity of females. Proteome analysis of mating pair combinations by 2-DE and LC-MS/MS showed that, of the 63 proteins identified, 38 were up-regulated and 25 were down-regulated. We have found some novel proteins related to fecundity including spermatogenesis-associated protein 5, PHD finger protein 7(NYD-SP6), agrinine kinase, actin-5C, vitellogenin, and ovarian serine protease nudel.

Keywords:*pesticides, Nilaparvata lugens, resurgence, mechanisms*

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Ecological engineering for pest management in rice: Reconciling on-farm biodiversity and food security

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Rice planthoppers (Hemiptera: Delphacidae) have exhibited outbreaks in many Asian countries in recent years despite escalating levels of insecticide use. This particular system reflects the broader issue of global food security whereby the capacity of agriculture to meet the needs of a burgeoning human population is being questioned. One response is to rely on ever more advanced technologies (e.g. genetic and chemical) to make farms more intensive. In contrast, ecological engineering aims to design production systems that are more compatible with and exploit natural ecological processes. This paper will present results from a three-year, multi-country assessment of the efficacy of ecological engineering for enhancing biological control of rice pests. Cultural techniques, especially the planting of nectar-providing plants on the bunds around rice fields, have been implemented in China, Thailand and Vietnam and experimentally compared with a control treatment consisting of normal farmer practice with multiple insecticide applications. Growing nectar plants on bunds has proven popular with farmers because crops such as sesame can be used and these provide an additional income. Results show that natural enemy numbers and diversity are markedly enhanced under ecological engineering and that this leads to effective control of rice planthoppers. Numbers of insecticide sprays required in the ecological engineering treatment were far lower than in the control yet planthopper densities were well controlled and rice grain yields maintained. One indication of the broader ecological consequences of ecological engineering is that numbers of frogs were increased.

Keywords:*biological control, Delphacidae, planthopper, nectar, parasitoid*

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Integrated Pest Management

Flowering plants improve biological parameters of rice planthopper natural enemies

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Enhancement of biological control by ecological engineering requires an understanding of the benefit to key natural enemies of candidate plant species. Free-choice tests by using a Y-tube olfactometer showed that adult Anagrus nilaparvatae and A. optabilis , the dominant egg parasitoids of rice planthoppers in China, were significantly attracted to flowers of Impatiens balsamena, Emilia sonchifolia and Sesamum indicum (sesame) compared with a clean air control. Equivalent experiments with adult mirid bug Cyrtorhinus lividipennis, an important egg predator of rice planthoppers, showed attraction to flowers of Tagetes erecta and Emilia sonchifolia. Female adult longevity of A. nilaparvatae and A. optabilis were significantly enhanced in the presence of sesame flowers compared to water whilst T. erecta, sesame, E. sonchifolia and T. procumbens extended longevity of C. lividipennis female adults. The numbers of brown planthopper (BPH) eggs in caged rice plants parasitized by A. nilaparvatae and A. optabilis were almost doubled in the presence of sesame flowers. Predation of BPH eggs and fecundity of C. lividipennis were strongly improved by sesame, whilst more BPH nymphs were consumed in the presence of E. sonchifolia. No obvious difference was recorded in the attack rate of A. nilaparvatae on BPH eggs in rice plants between without and with sesame flowers, whilst handling time (Th) was reduced by sesame flowers. Th of C. lividipennis to BPH eggs was significantly reduced by sesame and T. erecta. Results indicated that growing sesame, a long-flowering plant, on the bunds of rice fields could effectively increase biological control of rice planthoppers.

Keywords:*rice planthoppers, biological control, flowering plants, Anagrus parasitoids, Cyrtorhinus lividipenn*

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Comparative analysis of chemosensory system and metabolic enzymes of three rice planthoppers

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Chemoreception systems and metabolic enzymes are two most crucial evolutionary traits for insects. The capacity to rapidly adapt to resistant rice varieties and to develop resistance to various insecticides has led to severe outbreaks of the three rice planthoppers over the last decade. Nilaparvata lugens is a typical monophagous plant-feeder with rice and its wild cultivars as its hosts whilst Sogatella furcifera and Laodelphax striatellus are oligophagous herbivores with broader host ranges. Using the genome and transcriptomes of them, we profiled their peri-chemoreceptor system and the Glutathione S Transferase family. Significant reduction of gene number and distinct clusters in the evolution were observed in N. lugens. Rice virus infection increased the gene level and altered the host selection activity. GSTs exhibited different induction pattern among three planthopper species, and rice virus infection also increased their level, indicating an interaction between the vector and the virus. RNA interference of GST genes significantly increased the sensitivity of nymphs to insecticides. Double-stranded RNA-transgenic rice lines were constructed with the odorant coreceptor OR83b gene and a GST gene from L. striatellus, seperately. When nymphs fed on the transgenic rice plants, expression level of targeted OR83b and GST were reduced and the sense to rice plant and sensitive to insecticides were affected. This study provides the first elucidation of the peri-chemoreceptor system and GST gene family in a monophagous arthropod. The use of RNA interference and dsRNA transgenic rice illustrates the likely mechanisms for the olfaction of these insects, and the tolerance of them.

Keywords:*Planthopper*

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The genome of *Nilaparvata lugens*

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The brown planthopper, Nilaparvata lugens is the most destructive insect pest towards rice in Asia. In China, extensive outbreaks of rice planthoppers have been increasing in recent decades. These pests caused significant losses in rice yields through sucking, ovipositing and virus transmission (Cai et al., 2003; Wang et al., 2008). The BPH seriously threatens the world safety of food production. Regarding the influenced area and the people population, BPH is undoubtedly the most important agricultural insect pest around the world. Because of its long-distance migration, its quick adaptation to resistant rice varieties and development of high resistances to pesticides, N. lugens is difficult to adequately control. In this study, we present the draft 1,133 Mb genome of BPH, including its assembly, a set of 28,591 protein-coding genes. We also review findings of gene annotation efforts focused on genes related to migration and wing dimorphism, insect-plant interactions, symbiosis, detoxification, immune and virus transmission. The biological interpretation of the BPH genome provides a strong foundation for exploring the genetic basis of migration, of rice specialization and insect-rice interactions, of coevolved symbiotic associations and rice virus transmission, and of the pesticide resistance.

Keywords:*Nilaparvata lugens, genome*

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Progress in genome sequencing of the brown planthopper, *Nilaparvata lugens*

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The brown planthopper (BPH), Nilaparvata lugens (Stal), is one of the most serious destructive insects of rice in Asia. The long-term application of chemical insecticides results in development of resistance against them. Also, development of biotypes being able to infest resistant rice varieties has been a serious problem to be solved. Accumulation of knowledge of N. lugens in genomic level will provide us clues to comprehensive understanding of development of insecticide resistance and new biotypes, which leads to sustainable pest management technologies. Although we have sequenced BPH ESTs derived from various tissues or developmental stages through Sanger techniques, genomic information of BPH has been unavailable yet. But recent advance in sequencing technologies have resulted in a dramatic reduction of sequencing costs and a corresponding increase in throughput. The situation above mentioned motivated us to start genome sequencing of BPH. In this report, we introduce the current status of genome sequencing and relevant ongoing projects. The BPH genome sequencing is conducted in combination of pyrosequencing (Roche/454 Life Sciences GS FLX) and Illumina Sequencing technology. So far, total length of 454 long reads and Illumina short reads reached about 7.2Gbp and 67.1Gbp, respectively. As for transciptome data, RNA-seq using various tissues, stages and strains are in progress. In addition, the genome sequencing of yeast-like symbionts (YLS) using NGS technology also is ongoing. In order to allow BPH researchers to utilize BPH genomic data efficiently, we are developing the integrated BPH genomic database.

Keywords:*Nilaparvata lugens, genome, database*

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S1017TH20

Integrated Pest Management

Imidacloprid Resistance in the Brown Planthopper: Genes for nAChR Subunits and P450 Enzymes

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The brown planthopper (BPH) Nilaparvata lugens has recently exhibited remarkable resistance against imidacloprid, a neonicotinoid insecticide widely used for the chemical control of BPH. Insecticide resistance is usually caused by two main factors, namely, insensitivity to insecticides caused by gene mutation of insecticide target molecules, and high activity of the enzymes involved in insecticide detoxification. Gene mutation of the target molecule nAChR subunits has been reported in a laboratory-selected population of BPH. In contrast, field populations are reported to show high activity of P450 detoxification enzymes. We set out to elucidate the mechanism underlying imidacloprid resistance in BPH by using several resistant and susceptible strains. An [3H]imidacloprid binding assay did not show remarkable difference in binding level to membrane preparations of resistant and susceptible strains. In addition, comparison of the nucleotide sequences of nAChR subunits from multiple resistant and susceptible strains did not show common amino acid mutation sites between the two groups, suggesting that gene mutation is not currently involved in the imidacloprid resistance of field populations of BPH. In contrast, microarray analyses revealed that a P450 enzyme gene CYP6ER1 was commonly highly expressed in resistant strains. This gene has previously been reported to show high expression in other resistant strains, based on RNA-Seq data (Bass et al. 2011). The copy number of CYP6ER1 in the genome of resistant strains was estimated to be twice that of susceptible strains, suggesting that the high resistance level (~several dozen-fold) is mainly explained by the control mechanism of CYP6ER1 gene expression.

Keywords:*Nilaparvata, planthopper, imidacloprid, resistance, P450, nAChR*

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Sex ratio distortion by a male-killing Spiroplasma in the small brown planthopper, *Laodelphax striatellus*

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The small brown planthopper (SBPH), Laodelphax striatellus (Fallen) is a vector of rice and wheat plant viruses. We found the strongly female-biased sex ratio, i.e., 0.30 (proportion of males) in the Taiwanese (hereafter called the TW) and the slightly biased one, i.e., 0.43 in Japanese (the JP) strains. These strains harbored Spiroplasma, some strains of which are known to cause male-killing in some insect species. To determine whether Spiroplasma in the SBPH can manipulate their host reproduction, we carried out three examinations. First, Spiroplasma infection was examined by PCR in the TW and JP strains. The frequency of Spiroplasma infection was 0.86 in the TW strain, while it was 0.10 in the JP strain (N=50). Second, we established female-biased maternal lines (hereafter called FB lines) and non-biased maternal lines (NB lines) from these strains. Third, females of FB lines were treated with an antibiotic to eliminate the effect of symbionts. Tetracycline treatment cured the abnormal sex ratio of the progenies, strongly suggesting that symbionts manipulated their host reproduction. Since SBPH harbor Wolbachia (wStr), we established the maternal lines with single infection of Spiroplasma, which showed strongly female-biased sex ratio in their progenies. This is the first report of Spiroplasma-mediated-sex-ratio distortion in hemimetabolous insects.

Keywords:*small brown planthopper, Spiroplasma, sex ratio distortion, Laodelphax striatellus*

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Genes Regulating wing dimorphism of *Nilaparvata lugens*

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The brown planthopper (BPH), Nilaparvata lugens (Stal), is an important insect rice pest in Asia. Wing dimorphism is a special physiological phenomenon to this species. Resulted from physiological differentiation, muscle content and its development showed significant difference between macropterous and brachypterous adults, especially for the indirect flight muscle (IFM) which only exists in macropterous adults. We compared the proteome profiles between IFM and the trochanteral depressor muscle. The result showed that proteins related to cytoskeleton, energy production and conversion, fatty acid and lipid metabolism, transcription and translation were different between them. After expression verification and location experiment, two proteins, flightin and TpnC4 were found to be specifically expressed in the IFM. RNA interference and flight ability tests showed that flightin played key roles in thick filament assembling and sarcomere stability, and both of the genes were important to the performance of the flight system. Together, macropterous BPH use these key genes to realize the functions of flight muscle and capability of flight. These data provide a potential explanation for the wing dimorphism of BPH, and offered a baseline for further studies on muscle differentiation in other wing dimorphism species.

Keywords:*Nilaparvata lugens,wing dimorphism,proteome profile,indirect flight muscle, trochanteral depressor muscle,flightin,TpnC4*

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Integrated Pest Management

Paired genome sequence reveals insights into intimate symbiosis in brown planthopper

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A number of plant-sucking insects harbor endosymbionts which are thought to play an important role in the development of hosts. As one of the most serious pests on rice, the brown planthopper (BPH) Nilaparvata lugens (Stal) harbors both fungal and bacterial symbionts. In this research, we performed paired genome sequencing of both BPH and its obligatory symbiont, yeast-like symbiont (YLS) which were preliminarily thought to have capacity to provide nutrition for the host. Concentrated on several vital nutritional biosynthesis pathways, annotation of metabolism genes reveals that the BPH lacks the capacity for de novo synthesis of essential amino acids and genes underlying the urea cycle. Complementarily, the YLS is able to support essential amino acid synthesis and nitrogen recycling. Besides, the intimate symbiosis relationship is also reflected on sterol and vitamin biosynthesis in BPH.

Keywords:*paired genome, symbiosis, nutrition, brown planthopper, yeast-like symbiont*

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S1018F01

Integrated Pest Management S1018

Fifty years of monitoring insect migration in the United Kingdom at Rothamsted

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Rothamsted Research is the oldest agricultural research station in the world and has a long and illustrious history of studying insect migration, initiated by CB Williams in the 1920s. The ability to study country-wide spatio-temporal patterns of insect migration was greatly enhanced by the establishment of two national networks in the early 1960s. A network of 100 volunteer-run light-traps for sampling local macromoth populations was not designed specifically to study migration, but the spatial and temporal resolution of the data allows patterns of invasion to be monitored. The network of 12.2m high suction traps for sampling aphids and other small insects provides quantitative data on migration intensity and timing. These two networks have been complemented by continuous monitoring of high-altitude insect migration (between 150 and 1200m above the ground) by vertical-looking entomological radars since 1999. These three systems provide Rothamsted with an unparalleled capability for the study of insect migration. In this talk we discuss some recent results, with a focus on recently published work concerning pest aphids and moths, and Culicoides biting midges (the vectors of bluetongue and other animal viruses). Aphid phenology and abundance are affected by large-scale meteorological phenomena such as the North Atlantic Oscillation, and migratory patterns are better explained by aphid life-history traits than taxonomic relatedness. An important invasive moth pest (Autographa gamma) has been found further north in recent years and its population dynamics are dependent on a two-way migration. The flight season of Culicoides has been greatly extended over the past 40 years.

Keywords:*Migration, Monitoring, Radar, Trap, Aphids, Moths, Midges*

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S1018F02

Integrated Pest Management S1018

Management of Desert Locust

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The Desert Locust is one of the world's oldest pests and the most dangerous and feared of all migratory locusts in Africa and Asia. The Desert Locust Information Service (DLIS) at the headquarters of the United Nations Food and Agriculture Organization (FAO) in Rome, Italy operates a global early warning system in order to reduce the frequency and duration of devastating plagues that can affect one quarter of the world's populations and their agriculture. The system makes use of the latest scientific and technical advances to monitor the weather, ecological conditions and locust infestations on a daily basis, forecast their developments and provide early warning on the scale, location and timing of locust breeding and migration. National locust survey and control teams in two dozen frontline countries, working in some of the most remote and inhospitable places on this planet, record their field observations in a handheld device, eLocust2, that transmits the data by satellite in real time to analysts and decision makers. DLIS uses a custom geographic information system (GIS), one of the first developed in the world for operational pest monitoring, to manage and analyze the data with remote sensing imagery, seasonal predictions, historical locust data and models that estimate development rates and migration routes. During the past three decades, a number of lessons have been gained that can be applied to other migratory pest monitoring and early warning programs.

Keywords:*desert locust, early warning, remote sensing, GIS, FAO*

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S1018F03

Integrated Pest Management

Management of migratory *Helicoverpa* spp. moths in Australia.

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The two major pest species of Helicoverpa in Australia are H. armigera and H. punctigera. Both are capable of long distance migration, but despite considerable research, uncertainty surrounds the frequency, extent and patterns of migration. H. armigera has been characterised as a facultative migrant, leaving cropping areas only when conditions are unfavourable, and often overwintering there. H. punctigera, by contrast, has been regarded as an obligate migrant, breeding in winter on native hosts in remote areas of inland Australia and migrating to cropping areas in the south and east every spring, when these hosts senesce with the onset of hot, dry weather. These differences have been invoked to explain differences in seasonal phenology and tendencies to develop resistance to insecticides and transgenic crops. However, recent trends have cast some doubt on these assumptions. Eastern Australia, and particularly inland regions, was affected by an unprecedented drought from 2001 to 2008, and this may have changed the capacity of inland regions to support H. punctigera populations or the migratory behaviour of the pests. Similarly, extensive deployment of transgenic (Bt) cotton in the eastern cropping areas may have affected the season population dynamics of both species. Understanding medium to long term changes in the migration systems of these pests is crucial for developing forecasting techniques for the crops affected (including many grains, oilseeds and cotton), and for devising Resistance Management Plans for Bt cotton.

Keywords:*migration, Helicoverpa, cotton, forecasting, resistance*

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S1018F04

Integrated Pest Management

Monitoring and Forecasting Australian Plague Locust Metapopulation

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The Australian Plague Locust, Chortoicetes terminifera (Walker), is a widely distributed polyvoltine species with adapted mechanisms of diapause and quiescence, phase polyphenism, short-distance diurnal ranging and long-range nocturnal migration. It has 2-4 generations and frequently outbreaks which can cause severe damage to crops and pastures. Migration is crucial for its metapopulation persistence and plays an important role during plague. The Australian Plague Locust Commission (APLC) is responsible for monitoring Chortoicetes terminifera situation in eastern Australia, forecasting its population development and distribution, and carrying out control activities to minimise interstate migration. The APLC uses Normalised Difference Vegetation Index satellite images and rainfall distributions to monitor habitat conditions, and light-traps and fully-automated insect monitoring radars (IMR) to identify locust migrations in association with weather conditions and patterns. The APLC conducts routine ground survey within locust habitats, special follow-up in the region where migration is indicated by light-trap capture, IMR observed overflight, state- and public-report, and aerial surveillance when ground access is not practical or where a quick assessment of population level is required for searching suitable control targets. Thus the locust population development is simulated by a life-table model with localised locust and meteorological parameters and likely infestations are determined by a migration model which is based on wind trajectory analysis and locust orientation behaviours. The monitoring and forecasting of populations is essential for the APLC's decision-making and forms the basis of strategic control intervention. Migratory population exchange between the eastern and the western exists but is not significant or frequent.

Keywords:*Australian Plague Locust, Chortoicetes terminifera, Monitoring, Forecasting, Metapopulation*

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S1018F05

Integrated Pest Management

Understanding the Migration of *Helicoverpa armigera* with Radar and Modelling the Area-wide Population Dynamics in Northern China

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The cotton bollworm Helicoverpa armigera (Lepidoptera: Noctuidae) is one of the most serious biological disasters on multiple crops in northern China. Understanding the seasonal long-distance migration of H. armigera and accurately predicting the area wide population dynamics of this pest are essential to make effective control strategies. The seasonal high altitude wind born migrations of H. armigera have been observed with a scanning entomological radar and light traps in the past decade over both continent and the Bohai sea in northern China. The H. armigera moths migrated towards the north including over the Bohai sea at least as early as April, as soon as the moths had emerged from overwintering pupae and returned south on nights with northerly winds, or at altitudes where the wind was northerly during fall. Migrant H. armigera moths tended to fly at altitude where the wind direction, wind speed, and temperature were all optimum. A computer model has been developed based on the knowledge of biology and migration to simulate the population dynamics of this pest over a wide area in northern China. The model was validated with light-trap data and could potentially be used for forecasting the outbreaks of this pest and testing novel pest control strategies in northern China. This model also can be used to explore the resistance evolution in H. armigera to transgenic Bt crops in northern China.

Keywords:*Helicoverpa armigera, migration, model, population dynamic*

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Integrated Pest Management S1018

Radar observation of the migration of rice leaf roller *Cnaphalocrocis medinalis* Guenee in southern China

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The migration behaviors of Cnaphalocrocis medinalis Guenee in southern China were studied by using an 8.8mm wavelength scanning entomological radar, a searchlight trap and a conventional ground light-trap, field cages, systematic field survey and dissection of female ovarian in Xing'an Guangxi Zhuang Autonomous Region in China. The spatiotemporal distribution of landing areas of emigration population was analyzed using HYSPLIT, a software package that simulates migration trajectories. Results showed that the flight parameters of C.medinalis in southern China were different form that in eastern China. In southern China, C. medinalis started take-off behind 30min of sunset, reached the peak of take-off about 20:00, take-off can last 1-1.5h. C.medinalis generally flew below 1000m It can accumulate and form 2-3 layers dense layers when flew. The biggest density was at 400-500m. The insect layer was coincide closely with partial maximum wind speed and was not conincide with wind direction. However, the biggest density was not always at the height of biggest wind speed. Trajectory analysis showed that the population emigrating from Xing'an can arrive and survive at Liuzhou and Hechi in Guangxi Zhuang Autonomous Region, Huaihua and Xiangxi in Hunan province. Because of no suitable rice, the population arriving at Qiandongnan and Tongren in Guizhou province can't survive.

Keywords:*Rice leaf roller, Cnaphalocrocis medinalis Guenee, Radar observation, Flight parameters, Trajectory analysis*

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Integrated Pest Management

Overseas migration of the small brown planthopper, *Laodelphax striatellus*, in East Asia and its prediction

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Rice cultivars grown in Jiangsu province in eastern China shifted in 1990s from those of the hybrid rice to japonica types of good taste that were susceptible to the small brown planthopper, Laodelphax striatellus, and the rice-wheat cropping system prevailed there. Since then, its density had started to increase and peaked in 2005. And rice stripe disease infected by the insects with high Rice stripe virus viruliferous rate spread to the whole of the province. Consequently, overseas migrations in early June started to appear in East Asia, subsequently causing severe outbreak of rice strip disease in Japan and Korea. To notice possible immigrations in advance, a new prediction method for the overseas migration of L. striatellus from eastern China to Japan and Korea has been developed. The method consists of two techniques: estimation of emigration period in the source region and migration simulation. The emigration period was estimated with the calculation of effective accumulated temperature for the insect using real-time daily surface temperatures in the source. During the emigration period, migration simulations were performed two times a day, or at every dawn and dusk. The prediction method was evaluated with a cross-validation technique against four-year events from 2008 to 2011. The result showed that the emigration periods included the mass migration events in 2008, 09 and 11 as well as an emigration peak in the source in 2010. The method was successfully predicted those events, and the overall hitting ratio was found to be 93 %.

Keywords:*Laodelphax striatellus, overseas migration*

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Integrated Pest Management S1018

The spring migration of the small brown planthopper, *Laodelphax striatellus*, in eastern China

Baoping ZHAI[1], Yangyang Hou[2], Yuan HE[3], Yonggang Diao[4], Li Wang[5], Haiyan Zhang[6], Haibo Yang[7], Yue Zhao[8], Chao Han[9], Yanbo Xu[10]

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The spring migration of the small brown planthopper (SBPH), Laodeobhax striatellus (Fallen), were studied by field surveys in weeds, wheat and rice seedling fields, hourly collecting light-trap catches, ovarian dissection of macropterous females and trajactory analysis at four sites in eastern China in 2009 and 2010. The migration pathways of SBPH were simulated and analyzed by NOAA's HYSPLIT. The results were showed as follows: 1. The Spatial movements and dynamics of the spring population of SBPH in late May and the first half of June included long distance migration between different regions or overseas and local disperal among weeds, wheat fields and rice seedling fields. The adults dispersed from wheat fields to rice seedling plots just before wheat harvest, or when the taking-off SBPH were arrested by the weather conditions such as precipitation and strong winds. 2. The hourly collected light trap catches revealed that SBPH did migrate regularly after takeoff at dawn and dusk for a short or long journey, frequently, an all night fight. 3. The trajectory analysis illustrated that there were no definite spatial distribution for the source areas of the immigrant population of SBPH, so that both of the emigration and immigration could be seen in each research site and all of them were reciprocal source areas, which would be determined by wind fluctuation during the season.

Keywords:*Laodeobhax striatellus, spring migration, local dispersal, trajectory analysis, China*

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Do rain initiate the take-off of the White-backed Planthopper, *Sogatella furcifera* during night?

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The White-backed planthopper (WBPH), Sogatella furcifera (Horvath), is a major pest in rice production areas in China. It is also known as a typical migratory pest in Southeast Asia. Understanding of WBPH take-off behavior is critically important in studying the migratory processes of this pest, including source areas, migration pathways and landing places. In this study we focus on the relationship between WBPH take-off behavior and weather factors during night. Results suggested that: 1) WBPH take-off peaks occurred with rain during night; 2) WBPH undertake flights during dawn and dusk, and no take-off occurred during night without rain; 3) Total daily WBPH take-off number was not co-related with weather factors; 4) the sexual ratio was 1.2:1(female:male) during dawn take-off, and was 0.67:1 during dust take-off; 5) The night take-off rate was significantly positive correlated with relative humidity, and negatively correlated with temperature, and was not correlated with air pressure and vertical wind speed. These results suggested that further research on the mechanism to stimulate the ascent of WBPH was needed to better understand the take-off behavior during the emigration of WBPH in the field.

Keywords:*Sogatella furcifera, rainfall, take-off, emigration*

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Integrated Pest Management

Subtropical High constructed meteorological conditions of the migratory field of *Nilaparvata lugens*

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Using 27 yr's (1977-2003) light-trap data of Nilaparvata lugens from 18 stations, the correlation coefficients between the light trap catches and indexes of the Subtropical High over the West Pacific were calculated. In June and July, area and intensity indexes of the Subtropical High have positive correlations with light-trap catches from stations which located in the lower-middle reaches of the Yangtze River. In the lower-middle reaches of the Yangtze River, the damage caused by N. lugens is usually more frequent and serious than that in other regions. The immigration peaks of N. lugens in this region began in June, and the biggest peaks occurred in 16-20 July. Using CMAP and NCEP/NCAR Reanalysis data, climate background in June and July were summarized. When the ridge line of the Subtropical High stood around 25°N from 18 June to 10 July, rain-belts, which may force N. lugens to land, located in the lower-middle reaches in the Yangtze River (27-34°N), and low-level jet, which provided a high-speed passage for insect migration, occurred in 23-30°N. When biggest peaks of N. lugens immigration in the lower-middle reaches of the Yangtze River occurred in mid-July, the rain-belts stayed in Huang-Huai Valley (34-38°N), low-level jet expanded to 34°N. When the Subtropical High was stronger than normal in June and July, the precipitation in the lower-middle reaches of the Yangtze River was more than normal, and in South China was less, and low-level jet was stronger. In that case, the meteorological condition was more suitable for N. lugens migration.

Keywords:*Nilaparvata lugens, Subtropical High, migration, low-level jet*

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Integrated Pest Management

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Shift of outbreak area of meadow moth in China and the response to climate warming

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Meadow moth is an important migratory pest in China and could cause serious damage to crops and pastures in outbreak years. Meadow moth have experienced three outbreak periods since the establishment of the People's Republic of China in 1949. Distribution patterns of meadow moth were analyzed based on historical monitoring records. The results showed that the serious damage area of meadow moth had shifted northwards evidently from north China (the permanent breeding area) to northeast China during the past three outbreak periods and climate warming was the main cause for this phenomena through changing their migration and overwintering patterns. In 1950s, the soil moisture in north China was much higher than now that means a favorable condition in vegetation, which would facilitate adults in north China to stay locally. But since 1970s, climate warming has resulted in serious draught and advanced the emerging time of adults in spring which has resulted in a noticeable deviation of the adult stage from the rainy season. Both factors could stimulate adults to migrate northwards frequently since 1970s. Due to the limit of temperature, meadow moth in eastern part of inn Mongolia could not finish two integrated generations in normal years, so seldom overwintering cocoons could be found in history. But since 1990s, with climate warming intensifying, there have been a notable increase in occurrence frequency of the second generation larva in northeast China and consequently, an increase in overwintering cocoons. That provides plenty of local source for the spring population in northeast China.

Keywords:*Loxostege sticticalis, outbreak area, climate warming*

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Integrated Pest Management

S1018

Geographic Variation of Dormancy in the small brown planthopper, *Laodelphax striatellus* (Homoptera: Delphacidae)

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Photoperiodic stimuli induces nymphal dormancy in the small brown planthopper, Laodelphax striatellus(Fallen). A study was wade of the effects of temperature and photoperiod on the developmental patterns of SBPH populations samples from Hanoi, Vietnam and Jilin, China. Under a series of standard laboratory conditions of temperature and photoperiod, the incidence of dormancy and rate of larval growth differed significantly between JiLin and Hanoi populations. The incidence of dormancy was positively correlated with the latitude of the population source. The characteristics of the temperature and photoperiodic responses differed between Jilin and Hanoi, indicating complex evolutionary trends. The populations differed not only in the effect of temperature and photoperiod on the incidence of dormancy, but also in the effect of temperature on larval growth speed. And there was also a geographic clime in critical photoperiod for dormancy.

Keywords:*Laodelphax striatellus, geographic variation, photoperiod, temperature, dormancy*

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Integrated Pest Management S1018

A preliminary study on the behaviour mechanism of phototactic response of rice planthoppers

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The behavior characters of rice planthoppers around the trap lights were discussed in detail through systematic investigation of population density and female ovarian maturity types. 1, The immigration period of WBPH and BPH in Jinhua rice field were before 10th and 20th August; the emigration period were after 11th August and 4th September, 2011. 3, Whether in immigration or emigration period, Both WBPH and BPH tend to threw themselves on the upstairs lamp, this result was caused by the phototaxis lag effect of rhythm responding to a trap light. 6, According to the funnelling effect, the movement distance of focal panthoppers was much longer than the effective range of light traps during one night; they kept one straight line when flying to lamp in horizontal direction; when the illumination was less than 556 lx, panthoppers shew positive phototaxis, when more than 556 lx, they shew negative phototaxis, the typical phototactic response was with a "?"shaped catching curve. 7, Generally, most planthoppers flew against the wind in the breeze when they threw themselves on a lamp; wend speed was not too strong, they can flew down the wind; wind speed was too strong, light-trap catches were very little. 9, The number of light-trap catches in without moonlight night was much than that of in moonlight night, the greater contrast between light and dark surroundings, the more amount of light-trap catches.

Keywords:*rice planthopper, delay effect, funneling effect, illumination, phototactic behavior*

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S1019F01

Integrated Pest Management S1019

Modeling for insect pest management in fruit tree in Korea

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Pest management is a complex process including information gathering, decision making, taking action against a pest and evaluation of the action. In pest management system, the qualitative or quantitative modeling of the pest population help decision making. In Korean apple and pear, 10 species of pests are in heavy management concerns including fruit borers, hemipteran insects and mites. Population model can be useful in projection of population size or population events such as peak or most damaging stage occurrence, which will determine the management timing. Here we summarized some of the research works on population model development for each species, implementation of the model frameworks to users(farmers), and the gap between the ideal management system and current field management practices. From this, we present the area of further development for ecologically sound pest management.

Keywords:*apple, pear, oriental fruit moth, peach fruit moth, Grapholita dimorpha, degree-day model, decision*

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Matrix models for enhancing the role of biological control in IPM

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Models can provide an effective framework to guide for the implementation solutions for the management of insect pests in agricultural crops. Here I discuss two different applications of stage structured matrix models for enhancing the role of biological control in IPM. The first application focuses on opportunities to improve the success of classical biological control in the management of invasive insect pests through guiding selection of the most effective natural enemy species for introduction their regions of origin. This is illustrated using a stage structured matrix model for Epiphyas postvittana, a leafroller pest that recently invaded California. Using this approach, the early larval stage was identified as the most vulnerable point in the life cycle and consequently that Dolichogenidea tasmanica should be selected as the most promising classical biological control agent. The second application focuses on conservation biological control and the evaluation of population-level consequences of natural enemy exposure to pesticides. The majority of bioassays conducted to determine the selectivity of pesticides with respect to natural enemies are based on individual-level endpoint measurements. It is important to estimate the population-level consequences of the measurements from these life table response experiments, however, and an important approach for this is illustrated using a stage structured matrix model for Trioxys pallidus, a parasitoid of the walnut aphid in California.

Keywords:*biological control, matrix model, parasitoid*

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S1019F03

Integrated Pest Management

Modeling the spring emergence of *Grapholita molesta* in apple orchard

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The oriental fruit moth, Grapholita molesta (Busck) (Lepidoptera: Tortricidae) is one of important insect pests in Korean apple production. G. molesta overwinters as a full-grown larva within spherical and compactly woven larval-cocoons in the cleft of tree bark. In spring, the larvae develop to the pupa in the same place and adults emerge from late March or early April, then lay eggs on the tip of newly growing shoots of the host plant. Targeting management action on the egg and early larvae stages from the spring generation is critical to reduce the population of G. molesta during apple growing season. The model was developed to precisely predict the spring occurrence of G. molesta adults as a function of accumulated degree-days. Model validation was performed with the male moth catches in sex pheromone-baited traps in apple orchards in two major apple production regions (Uiseong and Geochang) of Korea in 2011. Five distribution models were applied to describe the cumulative proportions of G. molesta male caught relative to accumulated degree-days. The information-based approaches for analyzing observed data allow the data-based selection of a best model. The observed phenology of spring population of G. molesta was described by five models. The predicted dates for the cumulative 50% male moth catches were within five days variation. The performance of models was evaluated by statistical information criteria (AICC and BIC). The spring emergence model of G. molesta could be applied to determine optimal chemical treatment timing for controlling G. molesta in apple orchards.

Keywords:*Grapholita molesta, spring emergence, phenology, degree-days*

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Model simulation for IPM using DYMEX

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Population dynamics of greenhouse whitefly and American serpentine leafminer and pesticide application pattern of two-spotted spider mites (TSSM) were modeled and simulated using DYMEX model simulator. For population dynamics model simulation, leaf temperatures on reversed side of cherry tomato leaves were monitored according to three tomato plant positions using an infrared temperature gun. Air temperatures were monitored at same three positions using a temperature logger. The number of whitefly and leafminer were recorded by visual inspection and traps, respectively, to verify the performance of DYMEX simulation. A significant positive correlation between the observed and the simulated results was found when the leaf temperatures were incorporated into DYMEX simulation. This study demonstrated that the population dynamics of whitefly and leafminer were affected greatly by the leaf temperatures, rather than air temperatures in cherry tomato greenhouses. For modeling pesticide application pattern, we evaluated bioassay using TSSM in vitro. Four separated bioassay was evaluated at four different temperature conditions (20, 25, 30, and 35℃) with four commercial pesticides under commercial recommended dose. Mortality of TSSM were counted after 24 and 48 hours. A TSSM DYMEX model was developed and simulated under four temperature increasing scenarios (present condition, average 1, 2, and 3℃ increasing scenario) using DYMEX simulator. The DYMEX results showed that the pesticides application pattern were different among pesticides. In conclusion, the pesticide application should be changed for sound management of agricultural insect pest under temperature increasing scenario.

Keywords:*Leaf and air temperature, Temperature increasing scenario, Population phenology model, Pesticide application pattern*

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Development of the web-based information system for modeling insect population using site-specific weather data

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We developed the Agricultural Model Incubation System (AMIS) to support modeling research on plant diseases and insect pests. The system provides a web-based tool enabling users to run and verify their models using site-specific weather observation data or gridded weather data of 240m or 960m-grid size estimated for a certain region. The site-specific weather data is collected hourly from the public weather observation network. The gridded weather data is estimated from the site-specific weather data by using the geospatial interpolation method, Inverse Distance Weighting. Users can run models on hourly or daily basis and in various user-specific configurations by selecting time periods to run or by modifying model parameters. Output of the model run is provided to users in the form of maps or time series graphs, and downloadable through web browser. The system is capable of cohort-based simulation of insect population models using the site-specific weather data. At the moment, population models of 16 insect species, such as, Helicoverpa assulta, Grapholita molesta, Grapholita dimorpha, Myzus persicae, Sogatella furcifera, Laodelphax striatellus, Cnaphalocrocis dorsalis, Scirtothrips dorsalis, Unaspis yanonensis, Panonychus citri, Liriomyza trifolii, Trialeurodes vaporariorum, Bemisia tabaci biotype Q, Aphis gossypii, Frankliniella occidentalis, Spodoptera exigua, and forecasting models of 10 plant diseases are under verification and revision in the system. The verified models will be selected for application in web-based forecasting information systems. The system will be helpful for users to develop, validate, and analyze behavior of their models.

Keywords:*web-based system, insect population model, model simulation, site-specific weather data*

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IPM Module for the Sustainable Management of Sugarcane Stem Borer, *Chilo infuscatellus* (Pyralidae, Lepidoptera)

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Present study describes the evaluation of some environmentally sound tactics towards the management of sugarcane stem borer (Chilo infuscatellus). Different approaches were applied, Trichogramma cards, Detrashing, Light traps and Chemical control. The effects were found on the basis of incidence and intensity of Chilo infuscatellus. It was concluded from the results that the minimum infestation (8.71%) was recorded in T7 (Trichogramma cards + Detrashing + Light traps) which was statically at par with those of T3(Trichogramma cards + Light Traps), T1(Trichogramma cards + Detrashing) and T4(Detrashing + Chemical control) with increase in percent infestation 8.92%, 9.34% and 9.74% respectively. The treatment T8(Trichogramma cards + Chemical control + Light traps) showed 10.46% infestation was significant different from T6(Trichogramma cards + Detrashing + Chemical control), T9(Chemical control + Light trap +Detrashing) with infestation 11.66% and 11.86%. The treatment T2 (Trichogramma cards + Chemical control) with infestation 12.39% was statically at par with those of T5(Detrashing + light traps) with infestation (12.82%). The maximum infestation was observed in control treatment with 25.45%. The minimum infestation (11.66%) was recorded after 10 days interval while maximum infestation (12.53%) was observed after 20 days interval.

Keywords:*IPM, Sugarcane Stem Borer, Chilo infuscatellus, Trichogramma, Light Traps*

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S1019F07

Integrated Pest Management

Modelling population dynamics of pest insects in paddy field

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In this study, pest insects in paddy fields were analysed to characterize the relationships between occurrence patterns of pest insects and their meteorological factors in space and time using a self-organizing map (SOM). The pest insect data with nine meteorological factors in the Korean national scale from 1992 to 2008 were obtained from the Agricultural Production and Environment Information Service System. Nine pest insect species showed clearly temporal and spatial difference for 17 years from 1992 to 2008. Occurrence patterns of pest insects in paddy fields could be classified into five groups by the SOM learning process based on their abundance patterns. Clusters I and II were characterized by the higher densities of Chilo suppressalis and Pseudaletia separate, whereas clusters III and IV were identified by the higher densities of Lissorhoptrus oryzophilus, Cnaphalocrocis medinalis, Sogatella furcifera and Scotinophara lurida. The cluster V displayed the lower densities of nine pest insects. These different patterns were significantly related with the differences in meteorological factors including temperature, precipitation and evaporation and by changes in agriculture processes such as cultivation methods and chemical fertilizer. Finally, our results suggest that the studies on the spatial and temporal variations in pest population dynamics provides insight into the factors determining population behavior as well as providing guidelines for pest insects' control.

Keywords:*paddy field, self-organizing map, spatio-temporal dynamics, pest insects*

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S1019F08

Integrated Pest Management

Modelling complex interactions among organisms in paddy field

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The aim of this paper is to introduce a mathematical model for describing interactions among organisms ; paddy, weed, pest, natural enemy in a paddy field and to study a pest management problem in the paddy field. The model is basically a logistic, competitive, prey?predator model, predators being the pest and the natural enemy of the pest. For some pests, adult and nymph stage does harm for paddy, differently and so the model considers the pest population with two stages; immature and mature. This model will be applied to an integrated pest management problem to control pest populations in the paddy fiels, where release of sterile males and spraying of pesticide have been used as control measures for pest population. The sterile insect technique is one of the effective biological control for pests in the paddy culture system. Using optimal control theory, we show that the proper use of control measures might enhance the crop production of the model in an economically viable way. Using mathematical analysis, we have also found out an effective integrated pest management strategy.

Keywords:*Mathematical model, Integrated pest management, Sterile males, Pesticide, Optimal control strategy.*

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Rice Farm? An integrated virtual system to predict the effects of climate change on the rice-ecosystem

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RiceFarm is an integrated software system equipped with user friendly Graphic User Interface. The system is largely composed of three components. First one is the rice module. It is based on ORYZA 2000 which was developed by International Rice Research Institute in 2004. We modified ORYZA 2000 to accommodate to the virtual rice-ecosystem. Second one is the mathematical module which has the ability to predict the effects of climate change on the rice-ecosystem considering the interactions of spiders, pests and rice. In addition to the virtual ecosystem, RiceFarm included the socio-economic module. It enables us to produce the social and economic costs at advance caused by rice-ecosystem disturbance. We expect that RiceFarm would help to anticipate correctly the change of rice productivity according to the climate change and counteract to the change appropriately.

Keywords:*virtual system, rice-ecosystem, climate change*

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Catastrophe model and its application in insect ecology

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Because there are widespread phenomenon that is led to catastrophic by continuous movement in nature, especially in the insect ecosystem, this phenomenon is more common. It is difficult to explain the phenomenon by traditional description of the continuous movement of the calculus method. Authors had not only established the catastrophe model of the folding, the cusp, but also the swallowtail, the butterfly, and the elliptical in the study of insect population dynamics, and using these models to explain, describe and predict the population dynamics of pests. It has give theoretical foundation for agricultural production and application examples. This article reviews the application and problems of catastrophe theory in the field of insect ecology since catastrophe theory set up, and future development directions.

Keywords:*Catastrophe theory, Catastrophe model, Application, Population dynamic, Pest insect*

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S1019F11

Integrated Pest Management

Modeling strategies for pine wood nematode control

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Pine wilt disease is caused by the pine wood nematode Bursaphelenchus xylophilus, and the diseased spread by the pine sawyer beetle Monochamus altematus. Based on the individual beetle behavior, a mathematical model is proposed for evaluating control efficiency management of pine wilt disease in forest. The local rules regarding movement of vector populations (i.e., sawyer beetles) were applied on 2-D in the spatially structured models. Subsequently infested area was simulated in accordance with disease development. Population dynamics of pests was revealed in spatial and temporal domain. Intensity of control was further incorporated to the model. Asymptomatic carriers were considered in our model, the asymptomatic rate and nearest infestation distance were discussed through simulations. Finally, the control efficiency was predicted with different combinations of control areas and efforts. Optimized control strategy was further discussed.

Keywords:*pine wilts disease, control efficiency, individual based modeling, insect dispersal, disease spread*

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S1101M01

Insect Biological Control

Endophytic bacteria: Potential of biological and symbiotic control

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Endophytes are microorganisms that do not visibly harm the host plant but can be isolated from surface-disinfected plant tissue. They colonize an ecological niche similar to that of phytopathogens, and this fact might favor them as candidates for biocontrol agents. Many endophytic bacteria have been isolated from sweet orange, but our research has focused on the genus Methylobacterium, which occupies the same ecological niche as Xylella fastidiosa, the causative agent of Citrus Variegated Chlorosis (CVC). The genus Methylobacterium is described as a main player in the interaction between the endophytic community and the pathogen X. fastidiosa. Xylem-feeding leafhoppers are unique organisms in terms of their nutritional ecology; they are able to feed from xylem fluid. A clear association has been observed between Cicadellinae leafhoppers xylem-feeding habit and ability to transmit X. fastidiosa. A new genetic transformation tool, called paratransgenesis, has been used to prevent the transmission of pathogens by insect vectors. Paratransgenesis means genetic alteration of symbiotic microbes that are carried by insects. The overall strategy of disease prevention is called symbiotic control. The key to symbiotic control is finding a candidate microbe having an existing association with the ecosystem. Based on the premise of symbiotic control, we genetically modified the citrus endophytic bacterium Methylobacterium and evaluated its capacity to colonize a model plant and its interaction with X. fastidiosa. This work reports the successful transformation of Methylobacterium generate two different strains with a different gene each, suggesting a possible strategy for the symbiotic control of CVC disease.

Keywords:*CVC, endophytes, Methylobacterium, Symbiotic Control, Xylella fastidiosa*

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Insect Biological Control

Endophytic microorganisms : A review on insect control and recent advances on tropical countries

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Endophytic microorganisms inhabit plant tissues at least for a period of their life cycle, causing apparently no harm to the host. They constitute an alternative for the control of plant insect ?pests and diseases. They also help their hosts promoting plant growth and acting against stress conditions .Living inside plants, near pathogens, they are an ideal option to be improved by classical and molecular genetic methods. Many endophytic fungi and bacteria isolated from plant hosts are entomopathogenic . We have found these microorganisms as endophytes living in wild hosts from the Brazilian Amazon region and also from cultivated plant hosts. They were tested with success against insect-pests, cattle ticks and plant diseases from citrus, maize and eucalyptus among others. Also some endophytes were genetically modified, aiming to increase their protection against Citrus Variegated Chlorosis and other important plant diseases in Brazil.

Keywords:*ENDOPHYTES, BIOLOGICAL CONTROL*

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Fungal endophytes and their potential for biocontrol in cotton

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Asymptomatic fungal endophytes can provide protection to plants from a range of biotic and abiotic stressors. Included among these fungal endophytes are several species that are also known to be insect pathogens. Several fungal entomopathogens (e.g., Beauvaria bassiana, Lecanicillium lecanii and Paecilomyces spp.) can be both isolated from and inoculated to live systemically within a broad range of plants. As part of a survey of fungal endophyte diversity in cultivated cotton, we recovered a number of fungal endophytes that are known to act as antagonists against insects, plant pathogens, and nematode pests. In greenhouse trials, we successfully inoculated cotton seedlings using seed treatments of a field-collected endophytic strain of Paecilomyces sp. and a commercially available strain of Beauveria bassiana. In both cases, cotton aphid (Aphis gossypii) reproduction was significantly reduced on endophytically-colonized plants versus uninoculated controls in greenhouse performance assays. In a separate experiment, we tested for the endophytic colonization of plants cultivated under greenhouse conditions by B. bassiana when applied as a foliar inundative biocontrol agent to actively growing plants. We detected a high frequency (100-50%) of endophytic colonization of cotton leaves by B. bassiana for up to 21 days post-inoculation using both conventional culturing and a diagnostic PCR assay. Our results suggest that endophytic colonization of plants following the application of mycopesticides may be more common than previously realized.

Keywords:*entomopathogen, fungi, endophyte, microbial ecology, Aphis gossypii*

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Characterization and localization of novel proteins from *Antonospora locustae* (Protozoa: Microsporidae)

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The Microsporidian Antonospora locustae is an obligate intracellular parasite that exists in over 100 orthopteran species that is used as a biological control agent. In our study, we performed 2-deminsional electrophoresis of the Lammeli extracts of A. locustae spore walls, and separated 9 protein spots on the SDS PAGE. Then we extracted proteins from the gel and performed to mass spectrometry. Finally, three proteins have been identified after blasted in A. locustae genome database. One of them is a protein with 131 amino acid, and molecular weight at 26.24 kDa, and has a signal peptide, named as AlocSWP1. The gene for this protein corresponds to the open reading frame 1184 in the A. locustae genome database. This protein has no transmembrane domain, but has a Ricin B-like lectin motif. Experiment on developmental expression of the Alocswp1 gene showed that the gene starts to express on the 13th day, when the sporoblasts are forming, after inoculation within the fat body of locust. Furthermore, the anti-AlcoSWP1 labeled the surfaces of layers of sporoblasts, and sporont as well as spore walls, this further demonstrated that the protein starts to express when the sporoblasts form. Most interestingly that it started to express where a new layer to add onto previous one. Besides expression in spore wall, it was observed to express in polar tube. Based on the facts we proposed that AlocSWP1 may be the protein as jointers between the layers of the spore wall and polar tube.

Keywords:*Antonospora locustae, Spore wall protein, Polar tube protein, Immunolocalization, Jointer*

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Insect Biological Control

Preference of different fruit scents to the natal fruit fly, *Ceratitis rosa* in a choice trial

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Ceratitis rosa (Natal Fruit Fly) is one of the most economically important pests of citrus fruits throughout the world. C. rosa like other fruit flies' status as a major pest is maintained on cultivated fruits because it is more abundant in both cultivated and wild fruits throughout the year. C. rosa has a wide range of hosts including the citruses as one of the most preferred cultivated hosts. Fruit fly management aims at deploying Bait Application Technique (BAT) which has been considered to control fruit flies over a large area. This can be achieved by using the "Attract and Kill" approach incorporating attractants in the form of food and pheromones. In this study fruit scents will be used as attractants in a bait station to attract flies to the killing agent. Flies have been introduced to the different fruit scents in a choice experiment to determine the most preferred scent/s and preliminary results have shown that orange scent (Limonene) and banana scent (Isomyl acetate) are the most attractive to the Natal fruit fly. Further trials will be carried out to further test the attractiveness of these scents in fruit fly baits.

Keywords:*C. Rosa, fruit fly baits, attractants, fruit scents, fruit biocontrol,*

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Molecular pathogenesis of fungal entomopathogen *Metarhizium robertsii*

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In contrast to entomopathogenic bacteria and viruses, entomopathogenic fungi infect insects via cuticular penetration. Work to date has shown that highly active classes of enzymes, in particular proteases, lipases and chitinases, play major roles in penetration and subsequent liquefaction of the internal organs of the insect hosts. Our studies on the entomopathogenMetarhiziumrobertsii provides insights into the novel mechanisms by which pathogen evade host immunity by masking cell wall componentsusing a cell surface collagen-like protein and producing toxic cyclopeptide secondary metabolites in insecta.Theadhesins MAD1 and MAD2 encoded by the fungus are used to differentially attach to insects or plants. We also found a mammalianperilipin-like protein MPL1 is involved in control lipid storage, appressorium turgor pressure and virulence. After reaching insect hemocoel, the fungus was found to up-regulate an osmosensorMOS1 and a bacterial-like phosphoketolase MPK1to modulate fungal adaptation and propagation in insect hemolymph. The results of these studies facilitate the understanding of fungal entomopathogenicity and thus the development of cost-effective mycoinsecticides.

Keywords:*Molecular pathogenesis*

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Insect Biological Control

Microbial endophytes as source for genes related to biological control

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Researches for new molecules more effective against microbial diseases and pest control have been intensified in last few years due the development of the genomic and molecular biology approaches. This interest is also observed in obtaining pesticides with less environmental impact. In this context, endophytic microorganisms appear promising as a source of new species and therefore new metabolites. Studies showed that 51% bioactive compounds isolated from endophytic fungi have unknown chemical structure, thus, bioprospection of endophytes is considered an intelligent strategy to search for new compounds. Despite the increasing discovery of new compounds from endophytic fungi, there are a few studies involving knowledge of biosynthetic pathways responsible by their productions. Studies with endophytic bacteria Methylobacterium mesophilicum and Burkholderia cenocepacea and the fungus Epicoccum nigrum showed that these microorganisms are common inhabitant of plant tissues and extensive genetic variation can be found among isolates from a single location and host. Accordingly, to these characteristics these microorganisms are a source of new genes related to biological control of agronomic pests as well as production of new molecules that could be used for combinatory synthesis of many secondary metabolites. In order to identify these genes, we sequenced the genome of endophytic microorganisms and focused our studies in identification of genes related to polyketide synthases.

Keywords:*biological control, Microbial endophytes*

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From random release to risk assessment: Developments during the last twenty years

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Classical Biological Control (CBC) of insects with non-native natural enemies has been applied for more than 120 years, and the release of more than 2000 species of natural enemies has resulted in the permanent reduction of at least 165 pest species worldwide. Augmentative Biological Control (ABC) with non-native and native natural enemies are periodically released and used for over 90 years. At present, about 230 species of natural enemies are commercially available worldwide to control about 100 pest species. While thorough environmental risk evaluations were made for natural enemies of weeds, routine pre-release risk assessments were made only exceptionally for invertebrate biological control agents (IBCAs) used in ABC and targeted against arthropod pests. From current literature and experience it becomes obvious that intentional introductions of IBCAs against arthropods are in general safe and it appears that less than 1% has caused lasting adverse population effects in non-targets. During the last twenty years, legal frameworks to regulate introductions of exotic species were established by several international organizations. Guidelines on information requirements are provided to serve the preparation of dossiers to support applications and assist risk assessors and decision makers in a balanced risk/benefit evaluation. Risk assessment methodologies and tools, based on relevant ecological criteria, have been developed and applied during the last decades. In our presentation we will review the developments of risk assessment of non-native IBCAs during the last two decades and show how the currently proposed methodologies improve the safe use of non-native IBCAs.

Keywords:*biological control, non-native natural enemies, regulation of invertebrate biocontrol agents*

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Selection of non-target species and host specificity testing of natural enemies: Cabbage seedpod weevil (*Ceutorhynchus obstrictus*) parasitoids as a case study

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The assessment of natural enemies for biological control of pest arthropods has undergone a major change, particularly during the last 15 years. Concerns about the host specificity of several introduced natural enemies have resulted in the development of new frameworks for host range testing and calls for increased regulation of biological control, in order to confirm environmental safety of new agents. To evaluate potential impacts on non-target species we describe here a step-by-step procedure for selecting non-target species and for compiling a non-target species test list to assess the host specificity of new candidate entomophagous biological control agents. It is proposed that a set of categories, including ecological similarities, phylogenetic/taxonomic affinities, and safeguard considerations are applied to ecological host range information to develop an initial test list, ensuring that all aspects of potential impacts are considered. The initial list is then filtered to reduce the number of species to be tested by eliminating those with different spatial, temporal and morphological attributes and those species that are not readily obtained, thus unlikely to yield scientifically relevant data. The reduced test list is then used for a laboratory-based step-wise host range testing procedure that should allow over-and underestimation of the host range of a natural enemy to be avoided. On the basis of our own experience testing the host specificity of Trichomalus perfectus, a parasitoid of the cabbage seedpod weevil, Ceutorhynchus obstrictus, we discuss the practicality of the proposed procedure and the conclusions that can be drawn from the obtained data.

Keywords:*Biological control, non-target effects, Ceutorhynchus obstrictus, parasitoid*

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Measuring and predicting indirect non-target effects of new natural enemies

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The potential environmental impacts of biological control agents include not only direct effects, but also more subtle indirect ecological interactions, such as competition and apparent competition. It is very difficult to analyze and predict the outcome of such relationships based on pre-release testing in a quarantine facility. However, regulators sometimes require such data, and biocontrol practitioners must be prepared to address these issues in order to minimize ecological risk, and to obtain release permits. A framework for considering both top-down and bottom-up non-target effects is presented. Descriptive studies (using small arenas, focal observations, and molecular and biochemical tools) and manipulative experiments (including large cage trials and surrogate experiments) can complement historical, theoretical, and phylogenetic considerations to approach the goal of providing a comprehensive overview and prediction of the biocontrol agent's role in an ecological community.

Keywords:*biological control, non-target, indirect effects, apparent competition*

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Host range testing for a new biocontrol agent for *Uraba lugens* (Lep.: Noctuidae) in New Zealand

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Uraba lugens Walker (gum leaf skeletoniser) (Lepidoptera: Noctuidae) is an Australian pest of eucalyptus, now established in New Zealand. As a key part of developing classical biological control for this pest we conducted host range testing on its parasitoid Cotesia urabae Austin & Allen (Hymenoptera: Braconidae) to assess the risk it might pose to the New Zealand fauna. Host range tests were designed to examine physiological host range in the first instance, then, using choice tests and larger cage sizes, to increase ecological realism for the parasitoid. We found that although C. urabae preferred to attack U. lugens, it did show attack behaviour towards all of the eight species tested. However none of the non-target species supported successful development of adult parasitoids. Survival of parasitoid larvae inside attacked non-targets was limited to species from the most closely related subfamilies. We conclude that this parasitoid is unlikely to pose a significant risk to the few non-target species most closely related to U. lugens, and the risk to other species is extremely small. Based on this evidence, New Zealand regulators approved the application and C. urabae was released and successfully established in 2011.

Keywords:*Risk, Biological Control, Braconidae, Noctuidae, Host range testing*

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Regulations for release of biological control agents in Brazil: The case of *Cotesia flavipes*

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Although biological control takes place on a large scale and several exotic natural enemies have been introduced into Brazil, legislation of import and release was for a long time not fine tuned for biological control agents. Biological control agents were exposed to the same legislation, including the same registration costs, as chemical pesticides. Due to the activities of the recently established Brazilian Association of Biological Control Industry (ABcBio) the situation has changed and currently 9 microbial agents and 3 natural enemies (Cotesia flavipes, Trichogramma galloi and Neoseiulus californicus) are registered. The most widely used natural enemy in Brazil is the exotic parasitoid Cotesia flavipes for control of Diatraea saccharalis on 3 million hectares of sugar cane. Only 5 companies, serving 2.5% of the area of sugarcane, have applied for and obtained a registration for use of C. flavipes, the other companies are actually producing and selling the parasitoid "illegaly". Registration is still a long process, taking about 36 months and involves the ministries of health, agriculture and environment. The recent arrival of foreign biological control companies, together with the current collaboration between the three ministries and ABcBio is expected to result in more efficient, cheaper and quicker registration of natural enemies in the near future. Examples of registration used elsewhere in the world are used to streamline and adapt the Brazilian legislation and will include quality control measures.

Keywords:*regulation of biocontrol agents, Brazil, Cotesia flavipes*

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Environmental risk assessment of biological control agents in Canada: The cabbage seedpod weevil, *Ceutorhynchus obstrictus*, case study

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In Canada, risk assessment of classical biological control agents is required as part of the regulatory process. To justify release, a major emphasis is placed on host range studies to determine potential breadth of non-target species attacked and levels of attack on suitable non-target species that may occur. Cabbage seedpod weevil, Ceutorhynchus obstrictus is an invasive alien pest of canola, Brassica napus, in Canada. Trichomalus perfectus, a potential classical biological control agent, has been assessed for safety. Results indicate that T. perfectus may not be suitable for introduction.

Keywords:*biological control, weevil, parasitoid*

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The Mediterranean basin as source of invertebrate biological control agents for northern Europe: Safe "exotics"?

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In the past, Invertebrate Biological Control Agents (IBCA) released in European greenhouses in inoculative strategies were selected in northern European countries. Because of the mildness of climate, countries in the Mediterranean Basin are saw several of these IBCAs were able to establish. Several of these species used in inoculative releases have been considered risky for southern European countries. This problem, in combination with the recent limitations to the introduction of exotic natural enemies in Europe (and elsewhere) have prompted selection programs focused on IBCAs native to the Mediterranean Basin. Recent successes in biological control programs based on the use of these Mediterranean IBCAs (e.g. Eretmocerus mundus against Bemisia tabaci, Amblyseius swirskii against whiteflies and thrips, or Nesidiocoris tenuis against Tuta absoluta) and whiteflies) may have paved the way for future programs aimed at the selection of natural enemies safe for the whole western Palearctic eco-region. As they are native to Europe, these Mediterranean IBCAs could be safe for use in both northern European greenhouses (where permanent establishment would be improbable due to cold winters) andin the South (where they are already present). Because of the above mentioned reasons and the limited knowledge of the indigenous beneficial fauna occurring in the Mediterranean with many endemic species, studies focused on important groups of IBCAs in the Mediterranean Basin should be emphasized.

Keywords:*biological control, predator, parasitoid, climate, non-target effects*

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S1102M08

Insect Biological Control

Current regulations for import and release in Europe: How it is and how it should be

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Over the last 120 years, there have been more than 5000 introductions of around 2000 non-native species of biological control agents for the control of arthropod pests in classical biological control programmes. Another 250 species are commercially available for augmentative biological control. All these releases have resulted in sustainable, environmentally friendly pest management, with very few reports of any negative effect. Still, world wide some 25 countries have regulatory systems for biological control agents and many more countries are developing regulation. The European Union is not (yet) regulating the use of natural enemies. As a result, there is a "patchwork of regulation" across Europe in which some countries have strict controls on the import of non-native species, while other, often neighbouring countries have no restrictions on the import and release of "exotic species". Within Europe, the IOBC/WPRS Commission "Harmonized Regulation of Biological Control Agents" developed together with the European Plant Protection Organization (EPPO) a standard providing guidelines for the import, release and required application procedures for non-indigenous biological control agents. This standard is based on an Environmental Risk Assessment of natural enemies. Several European countries are now using this standard to evaluate applications for import and release of natural enemies. In this talk we will summarize recent developments and explain how The Netherlands coped with the evaluation of 150 natural enemies already in use and with the and with 39 applications for 21 new species.

Keywords:*exotic species, environmental risk analysis, regulation of biological control*

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S1103M01

Insect Biological Control

History and present status of insect biological control in Japan

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here are three basic strategies in biological control; classical biological control, augmentative biological control and conservative biological control. The first attempt of the classical biological control in Japan was the biological control of the cottony cushion scale. Serious agricultural pests were frequently introduced into Japan after opening the country in 1858. Classical biological control was very effective against those adventive pests, which are well controlled by introduced natural enemies now. The first attempt of introduction of forging natural enemies was that of the vedalia beetle to the cottony cushion scale in 1911. After this success we introduced several natural enemies to control new adventives pests, such as the citrus spiny whitefly, the woolly apple aphid the red wax scale and the arrowhead scale. Biological control of the chestnut gall wasp and tea spiny whitefly will be reviewed in this symposium. The first practical attempt of the augmentative biological control in Japan was mass release of Phytoseiulus persimilis to control spider mites in 1969. Several kinds of natural enemies are used in augmentative biological control in Japan now. Many greenhouse farmers are using predatory mites to control not only spider mites but also small insect pests. The current status will be summarized in this symposium. Finally, a case study of conservative biological control in Japan, such as effects of companion plants for control of aphids in Isahay reclaimed land will be reviewed.

Keywords:*Natural enemies, introduction, augmentation, conservation*

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Research on the biological control of insect pests in Korea

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Research and development of technology about biological control in Korea was reviewed roughly. In 19th couple of classical biological control attempts had made against invaded pest species such as the wooly apple aphid, Eriosoma langigerum, in 1934. From 1995 forward most of the research about biological control was on the use of natural enemy for the control of insect pests occurring in protected cultivation. The research papers about biological control which have been published until 2012 were total 158, since 1965. Major insect and mite pests of greenhouse crops include aphids, thrips, whiteflies, fungus gnats, mealybugs, spider mites, leafminers and scales. In cucumber 62 pest species, in pepper (including paprika) 64 pest species, in tomato 54 pest species, and in strawberry 52 species pest species of insect and mite are recorded in Korea. However, among the many pests only a few species are considered as key pest. In cucumber, cotton aphid (Aphis gossypii), greenhouse whitefly, Palm thrips (Thrips palmi), and cotton caterpillar (Palpita indica) are often cause damage. In pepper, oriental tobacco budworm (Helicoverpa assulta), garden thrips (Frankliniella occidentalis), green peach aphid (Myzus persicae), and cotton aphid (Aphis gossypii) are major pests. In tomato, greenhouse whitefly (Trialeurodes vaporiorum), sweet potato whitefly (Bemisia tabaci), and america serpentine leaf miner (Liriomyza trifolii) are considered as key pests. And in strawberry, two-spotted spider mite (Tetranychus urticae), cotton aphid (Aphis gossypii), and Palm thrips (Thrips palmi) are often reach economic threshold level. There are 127 items of natural enemy products enlisted in the listing

Keywords:*insect pests*

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Present status and future prospects of biological control in Thailand

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The diversity of biological control activities in Thailand ranges from basic biological information to the in time biological control of, mainly, the important agricultural pest insects. Such activities are made possible and substantial success accomplished through a national wide coordination. The exceptional current activities are in three streams: utilization of natural enemies for sustainable control of the outbreaks of cassava mealybugs complex, augmentative of biological control in black-headed caterpillars and coconut hispine beetles in coconut; and the management of pest insects for safety sugarcane production. Additional supporting research works include, utilization of natural enemies to control pest insects of economic crops; technology development of hymenopteran parasites, neuroptera lacewings, hemiptera and dermaptera predators and predatory mites, and, viability and package for transportation natural enemies and field release. With respect to the search for new agents, ongoing activities have been being facilitated the exploring, screening and efficacy test of the candidate endemic biocontrol agents. Consideration future prospects, besides the activities mentioned above, novel aspects of mass-production, packaging and utilization of those promising biocontrol agents, as well as, their shelf life for transportation prior to field release will be additionally investigated. One of the most important future prospects, without this the success of biological control program would not be achieved, is the acceptance of the farmers. Hence, demonstration plots, technology transfer and encouragement will be intensively conveyed to farmers to propagate natural enemies of their own.

Keywords:*biological control, future prospects, natural enemies*

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Review of classical biological control in Japan: A case study of chestnut gall wasp

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Chestnut gall wasp Dryocosmus kuriphilus, accidentally introduced from China, was a serious pest of chestnut trees in Japan. This pest was first found in Japan in 1941 and spread throughout the country until 1960s. Because no effective pesticides and native natural enemies were available, the pest had once been controlled by using highly resistant chestnut varieties. However, a new biotype of D. kuriphilus appeared in Japan and exploited even those varieties around 1960. In contrast, we found that D. kuriphilus seemed not to be a serious pest in China. Thus, it was expected that there must be some effective natural enemies in China. After a preliminary survey of the natural enemies in China, a specialist parasitoid Torymus sinensis was selected as a candidate for control agent of D. kuriphilus; 260 mated females of T. sinensis were released at Tsukuba, Ibaraki Prefecture in spring of 1982. D. kuriphilus population at the release site was almost completely suppressed by the introduced parasitoids in 1990s. T. sinensis steadily spread in Japan, and the damage by D. kuriphilus drastically decreased in many Japanese chestnut orchards. Therefore, the introduction of T. sinensis is regarded as a typical example of classical biological control in Japan. However, there appeared some ecological and taxonomical problems to be solved (e.g. displacement of the native parasitoid T. beneficus by the introduced T. sinensis).

Keywords:*Dryocosmus kurihilus, Torymus sinensis, classical biological control*

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Biological control of the camellia spiny whitefly, *Aleurocanthus camelliae*, in Japanese tea fields

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Camellia spiny whitefly, Aleurocanthus camelliae Kanmiya & Kasai, infestations have been rapidly increasing in the tea-producing districts of Japan since its first occurrence in Uji, Kyoto Prefecture, in October 2004 (Kyoto Plant Protection Office, 2005). Aleurocanthus camelliae closely resembles the citrus spiny whitefly, Aleurocanthus spiniferus (Quaintance) and has been mistaken for A. spiniferus (Kanmiya et al., 2011). Honeydew excreted by A. camelliae nymphs promotes the growth of black sooty mold on tea leaves, and contamination with sooty mold and pupal cases of A. camelliae reduces tea leaf quality (Kasai et al., 2010). Because nymphs reside on the undersides of leaves, spraying a sufficient number of nymphs with pesticides is difficult. However, A. camelliae has a parasitoid wasp, Encarsia smithi (Silvestri), which controls the citrus spiny whitefly A. spiniferus. Field investigations have suggested that once the density of A. camelliae decreases to a low level, this wasp can keep A. camelliae at a low density. Because the density of E. smithi is reduced by the spraying of nonselective pesticides, such applications can cause a resurgence of A. camelliae. Therefore, pest control systems that use selective pesticides on tea fields are important for the efficient control of A. camelliae. In this presentation, we show a control system for A. camelliae that considers the performance of E. smithi and the phenology of A. camelliae in Japanese tea fields.

Keywords:*Aleurocanthus camelliae, Encarsia smithi, pest control systems, selective pesticides, Japanese tea fields*

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S1103M06

Insect Biological Control

Where is the real IPM in Asian biological control? Importance of registration and patent in IPM.

Tetsuo Wada
Arysta LifeScience Corp., Japan

Once Japanese biological control researchers were criticized by European researchers that Japanese IPM research is isolated from practical plant protection world and they just complain the fact ; i.e. actual plant protection was dominated by chemical pesticides. Not by IPM, neither by microbials nor natural enemies…. But this was also true in the EU and USA situation until recently. IPM firstly started in the USA mostly in outdoor orchard in California. The concept of IPM is fabulous and around 1985 the California spray calendar did not mention any chemical pesticide names but only mention biological control and physical agriculture plant protection procedure. Since late 1980s, the center of biological control moved north western Europe, namely to The Netherlands where Wageningen/AmsterdamUniv. and private sectors harmonized to develop new biological control solution. 20 years after, basis of the pest control in the green house is dominated by biological control in EU. And in Japan, it is realizing after 17 years from the first registration of Encarsia formosa till recent Swirskii mite. Registration and patent are the key to enhance and promote biological control. No company is interested to non-intellectual property based technology. No more copying in Asia in chemical and biological pesticides but more original discovery and inventions in the IPM technology is real necessity in Asian IPM.

Keywords:*IPM*

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S1103M07

Insect Biological Control

Present status and future prospects of natural enemy industry in Korea

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Recently as consumer's concerns and needs for safe food increases, environmental-friendly farming which is known as not only the way to produce safe agricultural products in agriculture but also to protect the environment is getting more and more important. In environmental-friendly farming, it is the key technology to produce agricultural products without (or as less as possible) pesticide. Biological control based on natural enemy use is the most important pest control method in integrated pest management. The use of natural enemy for biological control is the fundamental technology to protect crop without chemical pesticide (eventually replace pesticide) or less pesticide use. Together with environmental-friendly farming, natural enemy industry has been developed and will be playing important role in the aspects. Now, Korean natural enemy industry has been developed much faster than the examples of other countries. But, except for the market leader Dongbu Ceres, most of the companies still remain at insufficient ability as a natural enemy producer, in the points of small scale of production, low level of technology, only few numbers of products, less capability of consulting for growers, etc. For further development of Korean natural enemy industry, it is required that such small natural enemy producers should make hard efforts on increasing their abilities on such aspects. It is expected that growers will produce safe agricultural products (that consumers are requiring) by the further development of natural enemy industry, and eventually environmental-friendly farming will be realized in much larger scale in Korea.

Keywords:*Natural Enemy industry, Dongbu Ceres, Korea*

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Commercial production of green lacewings, Mallada basalis (Walker) (Neuroptera: Chrysopidae) and its petty patent

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Due to the disastrous outbreak of the cassava mealybug complex during 2009-2010 in Thailand, the commercialization of the green lacewing, Mallada basalis (Walker) and increasing its use in biological control was a challenge. One of the reactions to the challenge is to reduce the cost of mass-production. Therefore, research on process for a feasible commercial mass-production of the green lacewing, was intensively investigated in three main experiments. The initial one was to study food source for adult. Mixture of honey: yeast (1:1) was the most applicable one, giving 646.67 eggs/adult/month. The later experiment was done on food source for larvae rearing. Eggs of rice moth, Corcyra cephalonica (Stainton) efficiently provided greater number of eggs compared with feeding aphids, administering a net reproductive rate (Ro, /) 4.56, finite rate of growth (I, / /d) 1.135, cohort generation time (Tc, d) 36.368 days and capacity of increase (rc, / /d) 0.127. The final experiment was the interval feeding of larvae demonstrated that larvae fed with 2, 3 and 4 g of rice moth eggs on days 2, 6 and 8 of the initial of larval development, respectively, gave an average of 260 pupae from 300 eggs and subsequent 241.33 individuals survived to adults. By following the process described, 1.25 million eggs/month with an approximate production cost 16 US$ per one thousand eggs were successfully achieved. In addition petty patent for the mass-production of the lacewing has been registered since 2010 (Petty Patent No. 1003000360).

Keywords:*green lacewing, Mallada basalis, commercial production, biological control*

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Fitness of the egg parasitoid *Trichogramma brassicae* is affected by size and shape of their host eggs

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Size and shape of host eggs could affect the fitness of egg parasitoids. We determined the fitness of Trichogramma brassicae, one of the most important egg parasitoids of numerous lepidopteran pests, using Plutella xylostella egg, which is flattened, ellipsoid and small (0.0237 mm3), and Trichoplusia ni egg, which is spherical and large (0.0634 m3). The parasitoids were separately reared using the eggs of each of the two host species. The treatments included a no-choice of the eggs of each of the two host species and a choice of the eggs of both host species. The fitness parameters assessed including size of the parasitoids (hind tibia length), adult longevity, fecundity, number of adults emerged, number of conspecifics emerged per host egg, duration of development, and sex ratio in the no-choice experiments, and parasitism and sex ratio in the choice experiments. In the no-choice experiments, the parasitoids reared from T. ni eggs were larger, lived longer, oviposited more, more conspecifics emerged per host egg, developed faster, and parasitized more than from P. xylostella eggs. However, sex ratios of the offspring were not different when the parasitoid parasitized both species. In the choice experiments, the parasitoids killed more but parasitized less P. xylostella eggs than T. ni eggs. The sex ratio of the T. brassicae offspring reared from T. ni eggs was similar to that from P. xylostella eggs. These results indicate that the fitness of T. brassicae more favored spherical and larger host eggs than flattened and smaller host eggs.

Keywords:*fitness, egg parasitoids, host shape, host size, biological control*

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Optimizing *Cotesia flavipes* (Cameron) release rates, and maximizing sugarcane yields for sugarcane borer complex in Thailand

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Infestations of the lepidopteran sugarcane borer complex, Sesamia inferens (Walker), Chilo tumidicostalis Hampson, Chilo sacchariphagus (Bojer) and Chilo infuscatellus (Snellen), the most important pest insects of Thailand sugarcane plantations, occur annually. Two consecutive field experiments were performed with Cotesia flavipes (Cameron), a braconid biocontrol agent of the pest, in Central Thailand, with two objectives: to optimize rate and timing of field release of C. flavipes in order to suppress the pest infestations and to maximize the yield of sugarcane. Demonstration sites, in each experiment, were done in four field locations, two acres at each site, and in completely randomized block design. Results in the first experiment came out with the higher rate of release, the higher percent parasitisation obtained. With 375 and 500 adults release/acre yielded 22.48±4.03 and 25.12±3.77% parasitisation which were significantly higher ($P<0.05$) than those release with 125, 250 adults/acre and in control treatment (non-release), 14.81±4.11, 17.16±3.37 and 2.56±1.47, respectively. However, percent parasitisation in treatments, 375 and 500 adults/acre, were not significantly different ($P>0.05$). Later experiment, timing of release, with 375 adults/acre, appeared that monthly release of the parasites in 1-month old sugarcane sprouts gave 25.46±2.13% parasitisation and the sugarcane yield was 38.38 tons/acre which were significantly higher ($P<0.05$) than those in treatments, monthly release in 4-month old sugarcane; monthly release in 4-month old plus cut-off the insect-damaged shoots of sugarcane; and control (non-release). Percent parasitisation in the four later treatments were 21.42±4.31, 25.46±2.13, 21.48±2.00, and 6.20±0.91; and yields gained were 13.55±0.83, 14.68±0.37, 13.48±0.87 and 10.63±1.22 tons/hectare, respectively.

Keywords:*release rate, sugarcane borer complex, Cotesia flavipes, parasitisation, biological control*

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S1103M11

Insect Biological Control

Utilization of natural enemies for sustainable control of the outbreaks of cassava mealybugs complex in Thailand

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Annihilating outbreaks of the cassava mealybugs complex unexpectedly occurred in cassava plantations, covering 224,000 acres, in every part, except Southern Thailand in 2010. Hence, a biocontrol project was immediately initiated challenging the sustainable control of these pest insects. Precursory monitoring of the cassava mealybugs complex in fields noteworthy revealed five species of the pest insects; Phenacoccus manihoti Matile-Ferrero (first record in Thailand), Phenacoccus madeirensis Green Phenacoccus solenopsis Tinsley Pseudococcus jackbeardsleyi Gimpel & Miller and Ferrisia virgata (Cockerell). Later, approximately 8,200 acres of cassava plantations infested by the mealybugs were promoted for the use of natural enemies. With a total of 54.63 million natural enemies in seven highly potential species, namely, Plesiochrysa ramburi (Schneider), Mallada basalis (Walker), Allotropa sp., Wollastoniella rotunda Yasunaga & Miyamoto, Amblysius longispinosus (Evans), Orius maxidentex Ghauri and Geocoris ochropterus (Fieber) were mass-produced. Dissemination and augmentative releases were attained. Subsequently, one hundred intensive training and technology transfer courses were provided for 1,758 cassava farmers' participation, to exhibit the application of the biocontrol agents. Additionally, four naturally occurring of the mealybug plots, eight acres each, were conducted to demonstrate the bicontrol applications, with paired treatments, release and unrelease (control) of the natural enemies. There was a significant lower population density of the mealybugs in treated plots than those in untreated plots. Final assessment of the project was accomplished both in the satisfaction, in terms of questionnaire, of the participating farmers and the control of the pest insects. Approach and details will be inclusively given in oral presentation.

Keywords:*cassava mealybugs, natural enemies, mass-produced, biocontrol*

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Conservation biological control of pest aphids by vegetation diversification: A successful example in potato fields of Japan

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Introduction: Vegetation diversification within farmlands by such methods as intercropping and companion plants naturally promotes pest suppression by enhancing the performance and abundance of natural enemies. Moreover, barrier plants reduce the immigration of pests into fields. Vegetation diversification within farmlands can a play plural role to suppress crop pests. In this study, the addition of barley strips were used as an example of vegetation diversification in outdoor potato fields of Japan. Methods: Three potato fields, two with barley strips and another of potato monoculture were prepared on reclaimed land in Isahaya City of Nagasaki Prefecture. Population dynamics of barley and potato aphids, and their natural enemies were investigated on barley strips and potato plants. Peak densities of potato aphids between conservation and monoculture fields and response of natural enemies between barley strips and potato plants were compared. Results: Barley strips supported different species of aphids compared to potato fields, but these different sets of aphids shared a common set of natural enemies. The amount of time between peak densities of aphid and their natural enemies was shorter in potato fields than in barley strips. The wingless and winged aphid populations in the field without barley strips was higher than in fields with barley strips, measured at peak aphid density. Conclusions: We have shown that barrier strips can potentially control aphids on potato plants by lowering colonization by aphids and by promoting larger and earlier immigration of aphid parasitoids and predators into potato fields.

Keywords:*Aphidius gifuensis, barrier plants, banker plants, monoculture, hyperparasitoids*

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Microbial control of cotton pests: Use of a naturally occurring entomopathogenic fungus (*Aspergillus* sp.) for managing *Creontiades dilutus* and beneficial insects on transgenic cotton crops in Australia

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The development and adoption of transgenic (Bt) crops that express the Bacillus thuringiensis (Bt) toxin has reduced the use of synthetic insecticide on transgenic crops to target Helicoverpa spp., the major insect pest of cotton in Australia. However, it has also increased the threat posed by sucking pests, particularly Creontiades dilutus (green mirid), which are unaffected by the Bt toxins in transgenic cotton crops. The aim of the study was to determine the efficacy of an entomopathogenic fungus Aspergillus sp. (BC 639) in controlling the infestation of transgenic cotton crops by C. dilutus and promoting interactions of transgenic cotton with beneficial insects. The results showed that application of BC 639 fungus controlled the number of C. dilutus adults and nymphs similar to plots treated with the recommended concentration of the commercial insecticide fipronil. The fungus was found to have minimal effect on predatory insects compared with fipronil and was most effective against C. dilutus when applied at the rate of 500 ml/ha (equivalent to 50 g spores/ha). At this rate, the fungus was as effective as fipronil for controlling C. dilutus populations and ensured the survival of predatory beetles, lacewings and spiders compared with fipronil treatment. The ability of the BC 639 strain to control C. dilutus infestations of transgenic cotton crops while conserving beneficial insect populations suggests its potential for supplementing integrated pest management programs to reduce the use of synthetic insecticides for transgenic cropping systems.

Keywords:*transgenic cotton, entomopathogenic fungus, integrated pest management, Bt, Creontiades dilutus,*

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Relative abundance of natural enemies of crop pests on *Bt*-transgenic and non-transgenic cottons in India

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To ensure a sustainable deployment of insect-resistant transgenic plants, it is important that they are compatible with the natural enemies of crop pests. Reduced survival of the Campoletis chlorideae was recorded on Helicoverpa armigera larvae reared on Bt-transgenic cotton leaves, but these effects were due to early mortality of the host larvae, and possibly poor nutritional quality of H. armigera larvae. There was no significant influence of Bt-transgenic cottons on larval period, larval survival and adult emergence of the ladybird, Cheilomenes sexmaculatus fed on Aphis gossypii reared on Bt cottons. Monitoring of Bt-transgenic and non-transgenic cottons for natural parasitization of eggs and larvae of H. armigera at farmer's fields from South-central India revealed that there were no significant differences in Helicoverpa eggs (19.8% versus 20.9%) and larval (7.4% versus 9.6%) parasitism on Bt-transgenic and non-transgenic cottons. Trichogramma spp. parasitized the eggs, while C. chlorideae and Eriborus spp. parasitized the larvae of H. armigera. The field studies on the effects of Bt-cotton on the nontaget natural enemies indicated that there were no significant differences in the numbers of C. sexmaculatus grubs and adults, Chrysopa spp. larvae, and spiders on Bt-transgenic and their counterpart non-Bt cottons. The numbers of generalist predators such as ladybirds, chrysopids, and the spiders were significantly more in unprotected than in insecticide protected Bt-transgenic and non-transgenic cottons, suggesting greater negative effects of insecticides on the natural enemies in comparison to Bt-cotton.

Keywords:*Bt-cotton, natural enemies, predators, parasitoids, abundance, Helicoverpa armigera, insecticides*

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Influence of transgenic *Bt* cotton on predacious arthropod assemblage and their impact on non-target pest suppression in Texas

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Bt-transgenic cotton adoption in the United States cotton belt has exceeded 90% during the last 8-10 years. However, in the Texas High Plains, the largest contiguous cotton patch in the world, Bt-transgenic cotton adoption is nearing 70%. Overwhelming adoption of Bt cotton and widespread planting of Bt corn have reduced overall lepidopteran incidence across the U.S. cotton belt, and have the potential to exert indirect effects on non-target insect species. Reaching an understanding of these effects is the logical first step in developing ecologically-intensive IPM programs for Texas cotton. While Bt-transgenic cotton adoption has improved lepidopteran control in the Texas High Plains, non-target insect pests such as thrips, cotton fleahoppers, cotton aphids, and Lygus are becoming increasingly important. We investigated the impact of Bt-transgenic cotton on field populations of cotton aphids, thrips, and cotton fleahoppers, as well as the cotton insect predator complex, in Bt-transgenic (PM 2326BGRR) and non-transgenic (PM 2326RR) cotton cultivars. In the two-year experiments, Bt-transgenic cotton demonstrated significant negative effects on cotton aphid and cotton fleahopper populations, but no significant effects on thrips or foliage-dwelling predatory arthropod populations.

Keywords:*Cotton IPM, Biological control, Transgenic Bt cotton*

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Honeybee foraging and pollination efficacy in Australian *Bt* cotton

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Although cotton is largely self-pollinating, previous researchers have reported that honeybees, Apis mellifera, can assist in cross-pollination and contribute to improved yield. Until the advent of GM (Bt) cotton, however, use of bees in cotton had been greatly limited by the excessive use of insecticides to control arthropod pests. Field trials conducted with honeybees in Bollgard II cotton crops in Australia over two consecutive years (using 8 and 16 hives/ha, respectively) showed that honeybees significantly increased cotton yield via increased boll set, mean weight of bolls, number of seeds per boll, and weight of lint per boll. Cotton flowers, and particularly cotton pollen, were not attractive to honeybees, and application of synthetic queen mandibular pheromone to flowers did not increase the level of bee visitation, nor boll set. Application of the insecticide fipronil at flowering against green mirid, Creontiades dilutus, in one trial severely reduced honeybee activity and its continued use will limit future opportunities for honeybees.

Keywords:*honeybee, pollination, Bt cotton, fipronil*

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S1104TU05

Insect Biological Control

Biological control of a cotton pest: *Bemisia tabaci* in Arizona transgenic cotton as a case study

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The adoption of Bt cotton reached 25M hectares in 12 countries in 2011. While the technology has proven to be highly selective to targeted pests, several pest groups have become more problematic in certain regions. That has not been the case in the western USA and it is instructive to explore why this is so using Bemisia tabaci, a key pest of cotton in the southwestern USA, as a case study. We have shown that natural enemies, particularly native generalist arthropod predators, inflict high levels of mortality on this pest in multiple crops and non-crop hosts and that predation is a key factor in governing the population dynamics of B. tabaci in cotton. Over the past 15 years, we have developed and refined an IPM system for B. tabaci and other key pests of cotton that has as its center the conservation of natural enemies and maximization of their biological control service. The fundamental components of our integrated control program include a rapid and accurate monitoring program coupled with robust economic thresholds and highly selective insecticides that are used only as needed to supplement biological control. The mechanisms underlying the success of our approach have been elucidated through the construction and analysis of detailed, field-based life table studies. Since 1996, when our IPM program was introduced and Bt cotton production began, insecticide use has dropped nearly 90% and producers have enjoyed over US$388 million in savings from pest loss and insecticide costs.

Keywords:*Biological control, sweetpotato whitefly, life tables, arthropod predators, integrated control*

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Ecology of *Trichogramma* egg parasitoids in Australian transgenic cotton landscapes and their utilization for biological control

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Helicoverpa (Hardwick) survivors are resurgent pests of Bt-transgenic cotton crops as they avoid transgenic tissue when feeding, especially when gene expression falters or wanes in the plant. Natural enemy impact is therefore advantageous when potentially disruptive conventional insecticides for Helicoverpa suppression are to be avoided. Trichogramma Westwood (Hymenoptera: Trichogrammatidae) egg parasitoids are the most utilised natural enemies in biological control programs worldwide. Generally, egg parasitoids fail to suppress Heliothine pests when mass released into cotton crops, primarily due to injudicious use of conventional insecticides, singly laid pest eggs avoiding detection, improved larval neonate survival at low densities, and asynchronous development benefiting pest over parasitoid. Despite this, relatively dense naturally occurring Trichogramma populations in the Ord River Irrigation Area (ORIA) of tropical northern Australia pervade local Bt-transgenic cotton crops and virtually negate Helicoverpa hatch. Although Trichogramma impact warrants inclusion in the local best management practice strategy for sustainable Bt-transgenic cotton production, reliance on conventional insecticides to suppress Helicoverpa survivors and other pests remains the status quo. When selective insecticides targeting secondary pests are applied judiciously, environmental conditions in ORIA cotton crops, especially early in the dry season, are conducive to Trichogramma proliferation and biological control of Helicoverpa is effective. There is scope, via habitat manipulation, to improve Trichogramma's suppression of Helicoverpa survivors in tropical Bt-transgenic cotton crops and there is evidence to support similar efforts in established Australian cotton growing regions.

Keywords:*Trichogramma, Helicoverpa, Survivors, Bt-transgenic cotton*

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Emerging pests and silver leaf whitefly; a challenge for Australian cotton systems.

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Silverleaf whitefly (Bemisia tabaci B-biotype, SLW) entered Australia in the mid 1990's and has progressively achieved pest status in all major cotton production regions. High adoption of Bt-cotton containing the Cry1Ac and Cry2Ab proteins has allowed dramatic reductions in pesticide use (from 8-12 down to <1) against Helicoverpa spp., the primary pest. Ironically, reduced spraying has allowed the green mirid (Creontiades dilutus) and green vegetable bug (Nezara viridula) to survive and they now require control (1-3 sprays). Control of these pests using broad-spectrum insecticides increases the risk of encouraging whitefly outbreaks. In addition, cotton pest management practitioners have noted that SLW were often more abundant in transgenic crops than conventional crops. Further, in small plot experiments okra leaf shaped genotypes appear to harbour fewer SLW. We compared SLW population development between Bt and non-Bt varieties, between okra leaf and normal leaf shaped varieties, and between sprayed (for mirids) and unsprayed systems. We found higher abundance of SLW on the non-transgenic cotton. Differences in SLW abundance between Bt and non-Bt in commercial crops are likely due to differences in spray regimes. Secondly, the okra leaf variety had significantly fewer SLW than the normal leaf cultivar, confirming valuable plant resistance. Finally sprayed cotton had higher abundance of SLW, indicating the detrimental effect on beneficial species. The results suggest a combination of okra leaf shape, transgenic cotton to reduce the need to spray, and use of selective mirid control will be options to help manage SLW.

Keywords:*whitefly, cotton, transgenic*

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Potential markets for stable industrialization of EPN business in Korea

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EPNs are important obligate biological control agents of insect pests and widely being used against a number of insect pests in several countries. Some of them are commercially produced in in vivo and in vitro. Although tasks to be worked out are still existed in commercial production and storage, marketing are also big issue for the development of EPN business. In Korea, commercial EPNs are being sold for the control of local and alien insect pests as the occasion demands. However, marketing has to be systematized for stable industrialization of EPNs. EPN markets should be expanded in these points. Many insect pests are occurred in greenhouse, propagation house of seedlings, golf courses, mushroom house, and so forth. Some crops or cultivating areas are restricted to apply chemical insecticides or hesitated to adopt chemicals by limited efficacies. For example, leaf beetles, sawflies, caterpillars, white grubs, fungus gnats, and many other insects are suitable targets to EPNs but sometimes cannot apply chemical insecticides by above reasons. The huge cultivating acreage of insect occurring crops or economic plants has searched for new alternative agents instead of chemicals. These may give big potential markets of EPN business in Korea. Thus, insects of important crops in each situation, efficacy of EPNs, and potentialities for EPN market will be discussed in this talk.

Keywords:*EPN, market, insect pests, biological control*

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The status of entomopathogenic nematodes in biocontrol: Ushering in new era

Ramon Khedo Georgis
Brandt, U.S.A.

The Status of Entomopathogenic Nematodes in BioControl: Ushering in New Era Georgis, Ramon. Brandt, 2935 S. Koke Mill Rd, Springfield, IL 62711 USA Progress in nematode commercialization during 1990's and 2000's was phenomenal. Development in large-scale production technology, quality control, proper label directions and easy-of-use formulations led to the successful commercialization of the nematodes as biocontrol products in selected markets against specific target insects. Despite this progress, the reality is that insecticidal nematodes have not reached their anticipated market potential. This is attributed mainly to competitions from new pesticide and biological chemistries, short shelf life, and lack of proper support from the distributors. In addition to the need to optimize nematodes virulents and shelf life, the development of nematodes for effective insect control in the context of sustainable agriculture will be a major challenge in new era. A truly integrated approach is required, in which all agriculture practices, including other insect control options, should be considered to obtain maximum effect.

Keywords:*Entomopathogenic nematodes, Biological Control, Commercialization*

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Wood borer control using entomopathogenic nematodes

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In recent years, the infestation of the wood borer is expanding in Japanese orchard and forest. Especially, Cherry treeborer (Synanthedon hecotor) infest to peach or cherry tree, Carpenter moth (Cossus insularis) infest to pear or apple and several other borers such as the long-horned beetles, ambrosia beetles damage and engrave heavily to the inside of wood trunk and it is not rare that the tree withers and dies. These wood borer infestations are increasing because of the change of cultivation system and management method, global warming and the increase of the orchard where the farmer give up cultivating. It is urgently needed to establish the effective control method. However it is difficult to success the enough efficacies by the chemical pesticide application because of the infestation area is inside of the wood trunk and the insufficient of distribution of pesticide to the gallery of wood borer which has complicated structure. Biosafe which is the entomopathogenic nematodes, Steinernema carpocapsae product in Japan is registered for many kinds of agricultural insect pests. Currently we have been proceeding the label expansion to the above serious wood borer species using the host finding ability of this nematodes species. In this presentation, the information about the application technologies and the field trials of several wood borer controls in our country are introduced.

Keywords:*wood borer, entomopathogenic nematodes, biological control, Steinernema*

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Competitiveness assessment through current market and R&D trend of entomopathogenic nematode

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Recent worldwide efforts for environmental protection have brought an impact on all agricultural fields. This has led to an awareness of urgent transition to eco-friendly farming, and biopesticides such as entomopathogenic nematodes are receiving remarkable attention as a new concept in crop protection. Hereupon, competitiveness assessment through current market and R&D trends of entomopathogenic nematodes are needed. Global biopesticides market size were valued at USD 1.075billion in 2010, and Korean market was valued at USD 37 million, which is roughly 3% of the entire global market. Considering biopesticides based on soil microbial products, natural fertilizer and eco-friendly organic materials controlled under fertilizer control act and eco-friendly farming act, the market size is estimated at around USD 300 million. Natural enemy market size in Korea is estimated at USD 25 million in 2010 and it is expected to grow to USD38 million by 2012 and USD 81.5million by 2016. Upon evaluating competitiveness of biopesticides among the entomopathogenic nematodes market in our country, it can be concluded that Korea possesses superiority and progressive factors when compared with existing foreign technologies in terms of commercialization, mass production, long term storage, wide host range, host searching, lethal effect and cost effectiveness from mass cultivation of entomopathogenic nematodes.

Keywords:*entomopathogenic nematode*

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S1105W05

Insect Biological Control

Production technology and field application of entomopathogenic nematodes in China

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Entomopathogenic Steinernema and Heterorhabditis nematodes (EPNs) are the biological control agents which have the specific capacity to actively search and infect insect larvae in the soil. Recent advance in the solid and liquid production technology of EPNs has greatly improved the yield, quality, and shelf life of these beneficial worms in China, and stimulated the field application of these nematodes for the control of different insect pests. The factors influencing the production efficiency were explored, including medium development, optimization of the culture parameters (gas supply, inoculum size, bacterial status, temperature, etc), recovery of the infective juvenile (IJ) inocula, formation of IJs, extraction and harvest of IJs. A company called Century Horse Development Ltd, under the guidance of Guangdong Entomological Institute, is running. Healthy and reasonable products in solid culture system are provided for field trials in China and for internal and international markets. Production cost is still a limiting factor for further commercial development in the world nematode-based market. Ideal pest targets for these nematodes were evaluated. Apart from the important insect pests (such as flee beetle Phyllotreta striolata, and chive midge Bradysia odoriphaga) in vegetables, new invasive pests such as the oriental fruit fly Bactrocera dorsalis, asiatic palm weevil Rhabdoscelus lineaticollis and banana moth Opogona saccchari (Bojer) were also controlled by EPNs. In addition, transgenic Enterobacter cloacae with the insecticidal tcdA1B1 from symbiotic bacteria of EPNs was constructed and used for termite control. The transgenic bacteria also showed high toxic potential against Spodoptera exigua, S. litura and P. striolata.

Keywords:*Steinernema, Heterorhabditis, Production technology, Field application*

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Entomopathogenic nematology in North America

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Research and commercial application of entomopathogenic nematodes in North America has a long history. In the pursuit of commercial viability, there have been a number of success stories, but also quite a number of dead ends. In this presentation, we provide insight into new opportunities for entomopathogenic nematodes that are on the horizon. Some novel targets that are currently being researched include a variety of wood boring insects, weevil pests, as well as pests of pollinators. Novel application methods and formulations that are being developed will also facilitate expanded use of nematodes as microbial control agents. Advances in biocontrol will also be enhanced through fundamental studies on the microbial ecology, epizootiology, trait deterioration, population dynamics and genetics of entomopathogenic nematodes, as well as basic studies on host-pathogen relationships.

Keywords:*Heterorhabditis, insect pests, Steinernema*

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S1106W01

Insect Biological Control

Bt Crops: Triumph of a creen technology based on *Bacillus thuringiensis*

Brian A Federici
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Over the past thirty years, numerous insect pathogens, including various viruses, bacteria, fungi, and protozoans have been evaluated as potential agents for controlling insect pests. Of these, only a few have been developed commercially for use in operational control programs. Baculoviruses, for example, were thought to have excellent commercial potential thirty years ago, but commercial use today is limited to a few special cases worldwide, on soybeans in Brazil, on cotton in Australia, on apples in the United States and Europe, and on potatoes in several countries in South America and northern Africa. Various fungi have also been investigated, but only recently have certain wild type and engineered strains of Metarhizium and Beauveria begun to show potential for use in controlling agricultural pests and mosquitoes. By far the most successful pathogens of insects developed commercially are strains of Bacillus thuringiensis (Bt) used for control of agricultural pests, primarily lepidopteran and coleopteran pests, and larvae of mosquitoes. Although successful, together all commercial bacterial insecticides constitute less than 1% of insecticide usage worldwide. In contrast to this, crops genetically engineered to produce Bt Cry proteins (i.e., Bt crops), mainly Bt cotton and Bt maize, first developed during the 1980's, now constitute a multi-billion dollar world market. These crops are grown on well over a hundred million acres in countries such as Australia, Argentina, the United States, Brazil, China, Korea, India, South Africa, and many others.

Keywords:*Bt Crops*

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S1106W02

Insect Biological Control

Replication and transmission of the sexually transmitted insect virus HzNV-2

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The nudivirus HzNV-2 (aka Gonad Specific Virus, GSV) is the only, sexually transmitted, sterilizing virus yet identified infecting insects. Virus replication occurs principally in the reproductive tissues of infected insects causing the malformation of these tissues and sterility of the infected moth. In females, virus replication causes the cells in these tissues to proliferate with hyperplasia of the common and lateral oviducts. Eventually, progeny virus accumulates in a plug blocking the reproductive opening of the female which serves as a source of virus contaminating males as they attempt to mate. Virus replication has additional ramifications in infected females which produce more mating pheromones and as a result attract more mates than healthy females. Males attracted to infected females then become contaminated during mating attempts. Clearly HzNV-2 has evolved genetic properties which function to manipulate the physiology and behavior of infected insects facilitating virus transmission and enhancing viral fitness. In order to identify viral genes responsible for these properties, we have sequenced the genome of HzNV-2. In the analysis of the genome of the virus three ORFs were found that had significant sequence homology to cellular genes not previously found in any viral genome. These included a juvenile hormone esterase gene, a gene coding for a putative a zinc-dependent matrix metalloprotease, and a major facilitator super family protein gene. These unusual virus genes are believed to play an important role in the underlying molecular mechanisms used by the virus to manipulate the host to enhance its fitness and transmission.

Keywords:*Virus, Sterilizing, Nudivirus*

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S1106W03

Insect Biological Control

The status and trends of *Bt* pesticides research in China

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The demand for biopesticides is rising steadily in all parts of the world. Public awareness about the environment is increasing, while pollution and health hazards due to synthetic chemicals are growing. Extensive and systematic research has enhanced the effectiveness of biopesticides, and as a result farmers and growers are using them in greater quantities.The economic value and ecological benefits make Bt attract peoples attentions and applied widely. In addition to the GMO , bio-pesticidesofBtisone of the most important andvaluable. New technique is promoting discovery of cry genes from native microbial resource. Hundreds cry genes were explored in recent five years. Status and trends of Btcry genes and pesticidesinChina will be introduced in the talk, as well asthe challenge and solutions in development of Bt bio-pesticides industry.

Keywords:*Bt pesticides*

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Possibility of *Lecanicillium* spp. for multiple pest control

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Entomopathogenic fungi have relatively high host specificity and can generally kill only one or a few species of insect pests. About 180 commercial mycopesticides have been registered world-wide using entomopathogenic fungi such as Beauveria bassiana, Metarhizium anisopliae and Lecanicillium spp. Their limited host range is one of the limiting factors for their commercial development. Recently it has been discovered that these fungi can also control plant pathogenic fungi such as Sphaerotheca fuliginea and Rhizoctonia solani. Previous studies demonstrated that an entomopathogenic fungal isolate could control either an insect or plant pathogenic fungus. Species of Lecanicillium are pathogenic to various host insects from caterpillar to aphids and whiteflies. At least 15 products based on Lecanicillium spp. have been commercialized. Three species of Lecancillium: L. longisporum, L. attenuatum and an unidentified isolate, have also recently been shown to kill 3 different species of aphids as well as suppress the growth and spore production of S. fuliginea in detached leaf disc bioassays. Under greenhouse conditions, a L. longisporum treatment reduced both cotton aphids and powdery mildew on cucumber leaves which were previously inoculated with aphids, powdery mildew or both. Through protoplast fusion of several isolates of L. longisporum, and L. muscarium, Japanese researchers have obtained Lecanicillium hybrids with multiple effects on soybean cyst nematodes, plant pathogens and insect pests, as well as colonization of soybean plant roots, making these strains promising for evelopment as broad spectrum biopesticides

Keywords:*Aphid, dual microbial control, entomopathogenic fungi, Lecanicillium, powdery mildew*

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Influence of synthetic media on mycelial growth and sporulation of the entomopathogenic *Aschersonia placenta* Berkeley & Broom

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Aschersonia placenta Berkeley & Broom (Deuteromycotina: Coelomycetes), an entomopathogenic fungal species, is host specific to some aleyrodids and scale insects. Very little is known about the biology and ecology of this fungal species. Collection of the fungus from orange orchards in the East of Thailand was done to determine its colony growth and sporulation on seven synthetic media, PDA (Difco), PDAM (Difco), SDAY (Difco), SDAM (Difco), CMA (Difco), V8 and WA (Difco), at 25±2 °C and 75±5 % RH for a period of 21 days. The experiment was done in a completely randomized design allocated in 5 replications. The result showed that the colony radial growth (in cm) on SDAY (2.04±0.13) and SDAM (2.09±0.10) was not statistical different (P>0.05), but they were significantly different from PDA (1.49±0.06) and PDAM (1.49±0.09) (P<0.05). Importantly, on PDA and SDAY the concentrations of spore suspension were 2.59x109 and 2.69x109 spores/ml, respectively, which were non-significant different (P>0.05), but they were significantly different (P<0.05). from PDAM and SDAM with 1.02x109 and 2.17x109 spores/ml, respectively. Also CMA, V8 and WA produced weak mycelia and sporulation; therefore, PDA and SDAY were the appropriate media for laboratory cultures of A. placenta.

Keywords:*Aschersonia placenta, synthetic media, colony radial growth, sporulation*

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Current status of microbial control using insect viruses in Japan

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The economic importance of microbial control agents is currently increasing in Japan. The most widely applied microbial control agent is the bacterium Bacillus thuringiensis, followed by entomopathogenic fungi and viruses. Among various insect viruses, baculoviruses are the only group used as microbial control agents at present. In Japan, two products based on baculoviruses are currently registered as biocontrol agents: one is a mixture of two granuloviruses (GVs) to control leaf-rollers (Adoxophyes honmai and Homona magnanima; Lepidoptera, Tortricidae) in tea fields, and the other is a nucleopolyhedrovirus (NPV) to control armyworm (Spodoptera litura, Lepidoptera, Noctuidae) on a variety of crops. These virus agents have been essential to control specific pests which have acquired resistance against chemical pesticides. The A. honmai and H. magnanima GVs are well adapted to control leaf-rollers in the tea system, and are also suitable for virus production using living insects. A notable feature of this host-pathogen system is that a single virus application was able to suppress the leaf-rollers throughout a whole year, during which four generations of the pest species normally occur in Japan. This was because the virus kills the host slowly, allowing active progeny virus to transmit itself to young insects in the next host generation. The NPV was originally registered to control S. litura on strawberry, lettuce, edamame (immature soybean) and soybean. However, its usage has broadened recently to include certain minor crops for which fewer registered chemical pesticides are available, such as shiso (Japanese basil), asparagus and edible chrysanthemum.

Keywords:*baculovirus, granulovirus, nucleopolyhedrovirus*

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Insect Biological Control

The negative control of crystal protein gene expression in *Bacillus thuringiensis* YBT-1518

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Bacillus thuringiensis forms proteinaceous crystals during sporulation that can account for 20-30% of the dry weight of the sporulated culture. There are many reports focused on the positive expression mechanism of the crystal proteins. But there is few on negative control. Here we present the negative control of crystal protein gene expression in B. thuringiensis YBT-1518 which produces rice-shaped protein crystals with 2 components of molecular mass 45 kDa (cry55Aa) and 54 kDa (cry6Aa) and are toxic to root-knot nematode. Previously, we found the expression of cry6Aa gene was negatively controlled by a gene named R2. While in other case, we isolated the third crystal gene cry5Ba (Guo et al., Appl Environ Microbiol. 74: 6997?7001, 2008). But in strain YBT-1518 we cannot observe the expression of Cry5Ba under the detection with an optical microscope and SDS-PAGE. After eliminating the plasmid which harbors cry55Aa and cry6Aa, there is not effect on the expression of Cry5Ba. RT-PCR detection showed a transit transcription at the onset of the stationary phase. More than 10kb flank sequences (each side) of cry5Ba can not be found to affect the expression of Cry5Ba. After finishing the genome sequence and bioinformatics analysis, a gene locus BMBsr1 which reversely complements the ribosome binding site of cry5Ba was predicted as a sRNA coding sequence. By gene knock-out and heterologous expression, gene BMBsr1was found to inhibit the expression of Cry5Ba in strain YBT-1518. When applying gfp and lacZ as report marker, BMBsr1 was found gene silencing effect either.

Keywords:*crystal protein*

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AcMNPV infection suppresses the expression of host immune and juvenile hormone-regulated genes in *Spodoptera exigua* larvae

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The impact of Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) infection on host gene expression in Spodoptera exigua 4th instar larvae was investigated through the use of 454 sequencing-based RNA-seq of cDNA libraries developed from insects challenged with active AcMNPV or heat-inactivated AcMNPV. By comparing the two cDNA libraries, we show that 201 host genes are significantly up-regulated and 234 genes are significantly down-regulated by active AcMNPV infection. Down-regulated host genes included genes encoding antimicrobial peptides, namely three gloverin isoforms and an attacin, indicating that the viral infection actively repressed the expression of a portion of the host immune gene repertoire. Another interesting group of down-regulated host genes included genes encoding two juvenile hormone binding proteins and a hexamerin, all of which are involved in juvenile hormone regulation. The expression of these genes was enhanced by the topical application of Juvenile Hormone III (JHIII) in the insects challenged with heat-inactivated AcMNPV. However, infection with the active virus strongly suppresses the expression of these three genes, regardless of the absence or presence of JHIII. Using RNA-seq, we have identified groups of immune-regulated and juvenile hormone-regulated genes that are suppressed by infection with active AcMNPV. This information and further studies on the regulation of host gene expression by AcMNPV will provide the tools needed to enhance the utility of the virus as an effective protein expression system and as an insecticide.

Keywords:*AcMNPV, Spodoptera exigua, comparative transcriptome, host gene regulation, host gene suppression*

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Events of mycosis hotbeds in Colorado beetle populations in the South Urals from 2008 to 2011

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Biocontrol of pest insects is one of the main branches of modern science research. Entomopathogenic fungi take up significant place in this issue. Colorado potato beetle was found in Bashkortostan in 1976. Mass infection by entomopathogenic fungi was found in 2008 for the first time. Analysis of level of infection was started in this year. 76 excerpts from 33 districts were assayed from 2008 to 2011. Beetles with manifestation of mycosis were taken post mortem during toxicological assay. Maximum value of infected individuals (80%) was observed in 2009 year in west region, whereas maximum values in 2010 and 2011 years were 30 and 38 %, recently. Hotbeds were noted in central and north regions. Isolates were obtained from Colorado beetle cadavers collected in all hotbeds. Isolates refer to genus Beauveria. A total of isolates were characterized by RAPD-PCR. Percentage polymorphic loci (P), Shannon's information index (Is), Nei's genetic diversity (h), total genetic diversity (Ht), genetic diversity within group (Hs) and the relative magnitude of genetic differentiation among groups (Gst), genetic identity (I) and genetic distance (D) were calculated for all pare-wise combinations of groups. Dendrograms were constructed by cluster analysis based on the unweighted pair group method with arithmetical means (UPGMA). Our investigation shows degree of phylogenetic relationship between different isolates of enthomopathogenic fungi from population of Colorado potato beetle in the South Ural. Investigations funded by Russian Foundation for Basic Research 11-04-01886_a and 11-04-97022-r_povolzye_a.

Keywords:*Colorado potato beetles, mycosis, RAPD-PCR*

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Recent studies on the thermotolerance of entomopathogenic fungi

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For successful commercialization of entomopathogenic fungi a problem of low thermotolerance must be overcome. To increase thermotolerance the effects of nutritional manipulation of culture media and oil-coating of conidia of Beauveria bassiana and Metarhizium anisopliae isolates were investigated, followed by pairing of similar isolates. Millet grain and whey permeate-based cultures produced more thermotolerant conidia than ¼SDAY cultures. Conidia produced on millet or whey permeate were more hydrophobic and exhibited greater thermotolerance than those produced on ¼SDAY. The mycotized millet grains were further coated with plant oils. Conidia with a corn oil coating were superior to sunflower- and cotton seed oil coatings in thermotolerance. Pairing of two B. bassiana isolates was done to generate new colonies with enhanced thermotolerance by possible transcriptional alteration. One colony with completely different morphology was isolated from the paired culture and it had the highest thermotolerance without significant loss of virulence. Recently, thermo-susceptible B. bassiana mutants were generated by a fungal transformation platform to discover thermotolerance-related genes, which are now under determination. These results suggest that the thermotolerance of entomopathogenic fungi can be increased by the nutritional manipulation, oil-based coating and pairing. Genetic information on fungal thermotolerance is going to be clear in near the future.

Keywords:*entomopathogenic fungi, thermotolerance, Beauveria bassiana, Metarhizium anisopliae*

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Identification of three new Cry proteins synthesized by *Bacillus thuringiensis* subsp. *jegathesan*

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Bacillus thuringiensis subsp. jegathesan is known to produce at least five 'mosquitocidal' proteins ? Cry11B, Cry19A, Cry24A, Cry25A and Cyt2B. Among these, however, Cry11B is the only protein that shows high larvicidal activity, indicating that there could be other unknown mosquitocidal proteins produced by this bacterium. Through mass spectrometric analysis of proteins separated by polyacrylamide gel electrophoresis, three additional Cry proteins were discovered in this strain. PCR amplification and sequencing of their coding genes revealed that each was encoded by a novel gene, which were designated, respectively, as cry30Ca2, cry60Aa1 and cry60Ba1. Interestingly, cry60Aa1 and cry60Ba1 occur as an operon, and were also found in B. thuringiensis subsp. israelensis (ATCC35646). When these Cry proteins were produced using the cyt1A-p/STAB system in acrystalliferous B. thuringiensis 4Q7, Cry60Aa1 and Cry60Ba1 assembled into one large crystal per cell, whereas Cry30Ca2 did not form a crystal. The median lethal concentrations (LC50s) of Cry60Aa1, Cry60Ba1, and Cry60Aa1+Cry60Ba1 were 5.5, 7.9 and 2.9 μg/ml, respectively, when they were tested against early 4th instars of Culex quinquefasciatus. In addition, SDS-PAGE analysis indicated that co-synthesis of Cry60Aa1 and Cry60Ba1 may interfere with each other's synthesis by an unknown mechanism.

Keywords:*Bacillus thuringiensis, Cry30Ca2, Cry60Aa1, Cry60Ba1*

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Nasonia genomics and the genetic improvement of parasitoids for biological pest control

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Parasitoids are used widely in biological control of insect pests. One method is augmentative release, which involves mass-rearing of parasitoids and release into human modified habitats (e.g. crop fields, greenhouses, barns) to suppress pest numbers. However, these methods mostly use "wild" parasitoids. An approach for the future will be to "domesticate" parasitoids for improved biocontrol agricultural systems. To achieve this goal, a better understanding of the genetics and genomics of parasitoids is needed. Genomes of three related parasitoids in the genus Nasonia have recently been sequenced. Promising traits for parasitoid domestication are discussed, along with findings from genomic and genetic studies of Nasonia relevant to the issue of parasitoid improvement in biological control.

Keywords:*genomics, parasitoids, pest control*

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Genetics of hymenopteran sex determination

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Sex determining mechanisms are bewildering diverse and turn over rapidly in evolution. Sex determination in insects occurs through a cascade of genetic switches with conserved genes at the bottom but strong diversification in the primary signal genes. I will discuss the genetic regulation and phylogenetic pattern of sex determination in the insect order Hymenoptera with specific focus on consequences for mass culturing and biological control aplication. All hymenopterans have haplodiploid reproduction (haploid males from unfertilised eggs, diploid females from fertilised eggs), but with different underlying genetic pathways of sex determination. Single locus complementary sex determination (slCSD) is known from >60 species in different families including the parasitic Ichneumonoidea. Within the family Braconidae slCSD and multi-locus CSD occur in closely related species. Maternal effect genomic imprinting sex determination is only known from the chalcidoid Nasonia. Endosymbionts and genetic elements that alter host reproduction and sex allocation occur widespread among Hymenoptera. They can induce cytoplasmic incompatibility, parthenogenesis, son killing and feminization and could potentially be exploited in biological control.

Keywords:*biological control, hymenoptera, sex determination*

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Evolution and genomic architecture of host specificity in aphelinid parasitoids of aphids

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Assessing the risks of post-introduction evolution in host specificity of parasitoids introduced for biological control is crucial for their safety. Using research on the host specificity in Aphelinus species proposed for introduction against aphids, I discuss three approaches to assessing the risks of post-introduction evolution. The first approach uses phylogenetic and phylogeographic patterns in host specificity. However, studies using this approach showed that host specificity is not phylogenetically conserved and that variation in parasitism among populations within a species suggests adaptation to locally abundant aphids. The second approach is to determine the genetic architecture of host specificity. Interspecific crosses and backcrosses to map quantitative trait loci (QTL) affecting parasitism of a novel host species showed that changes in a few QTL were involved. We are now doing whole genome scans for evidence of local hot spots of differentiation associated with variation in host specificity. The third approach is to determine rate of evolution either in laboratory experiments or after biological control introductions. Laboratory selection for parasitizing a rarely used aphid species gave a more rapid shift in host specificity than expected. Using crosses and backcrosses of high versus low selection lines, we are mapping the QTL involved in the shift. We are also testing whether such evolutionary shifts have occurred in Aphelinus introduced for biological control, and if so, the genomic architecture of such shifts.

Keywords:*host specificity, genetics, genomics, evolution, phylogenetics, phylogeography, reduced representation libraries, next generation sequencing, SNP, QTL*

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Global analysis of the transcriptome of teratocytes reveals its function in development of *Cotesia vestalis* larvae

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Teratocytes are cells that originate from the serosa membrane of some hymenopteran parasitoids, which are released into the host haemolymph during the hatching of parasitoids eggs, and develop in the host haemolymph. They are considered to be involved in parasitoid larval nutrient uptake, host immunosuppression and host manipulation. The endoparasitic wasp Cotesia vestalis develops in the hemocoel of larval stage of their host Plutella xylostella with the help of teratocytes. We use the Illumina method to sequence the transcriptome of Cotesia vestalis teratocytes. In a single run, 6,967,361 sequencing reads have been produced, and these reads were assembled into 24,165 unique sequences with a mean size of 208bp. The BLASTx E-value of 79% unigenes ranged from 1.0E-5 to 1.0E-50; the similarity of 44% unigenes against homologous sequences ranged from 60% to 80% and 47% homologous sequences are from Drosophila, Apis, Anopheles and Nasonia species. The putative functions of teratocytes were predicted by gene annotations: (1) nutrient metabolic which consisted of 3 pathways, host fatbody digestion, host trehalose metabolic and nutrient secretion; (2) host regulations which consisted of 6 pathways: host reproductive system regulation, host juvenile hormone regulation, host ecdysone regulation, host chitin regulation, host protease inhibition and host tissue injury; (3) host immunosuppression; (4) toxin-related function; (5) anti-microbial function, and (6) microvilli construction-related function. Funding for this study was provided jointly by the National Science Foundation of China (No. 30871675), Zhejiang Provincial Natural Science Foundation of China (No. Z3100296) and NSFC Innovative Research Groups (No. 31021003).

Keywords:*teratocyte, transcriptome, development, Cotesia vestalis*

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Combining symbiont-manipulated phenotypes with genomics to unravel genetic bases of parasitoid life history traits

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Arthropods, among which parasitoid wasps are no exception, are frequently infected by vertically transmitted microorganisms that deeply impact their phenotypes. Different traits can be affected including reproduction, physiology and behavior. These symbiotic manipulations often expand phenotypic variation largely beyond standing nuclear genetic variation. By studying the molecular mechanisms underlying these interactions, we may expect to gain insights on the genetic bases of the affected traits, in a way very analogous to the study of genetic mutants. This framework led us to develop genomic and transcriptomic approaches on two symbiotic systems involving drosophila parasitoids: - In the solitary parasitoid Leptopilina boulardi, the virus LbFV forces infected females to accept to oviposit in already parasitized larvae, a behaviour known as superparasitism. Such manipulation is beneficial to the virus as it benefits from horizontal transmission when parasitoids share the same Drosophila. - In Asobara tabida, removing of Wolbachia leads to copious apoptosis during oogenesis and to a dramatic reduction in egg load. Furthermore, aposymbiotic eggs do not develop, thus rendering Wolbachia necessary for insect reproduction. On these two systems, sequencing of the genome of the symbiont is in progress as well as global transcriptomic approaches comparing infected and uninfected individuals. The first results on these two systems will be presented.

Keywords:*genomics, transcriptomics, superparasitism, oogenesis, symbionts*

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Insect Biological Control

The genetics and genomics of reproductive decisions in parasitoid wasps

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Reproductive decisions in insects are of profound interest to evolutionary biologists and ecologists, and are also crucial to attempts to promote or hinder populations of beneficial or pest insects in the wild. In many cases though, our understanding of reproductive behaviour is limited to either the phenotype, or the genetic basis of reproduction in a very limited group of model species (e.g. Drosophila). The advent of high-throughput genomic technologies has the potential to revolutionise our understanding of insect reproductive behaviour, particularly in species with limited genetic resources (i.e. virtually all insects). Here I will describe our on-going work to explore the genetic and genomic basis of reproductive behaviour in the parasitoid wasp Nasonia vitripennis. I will focus on sex allocation and female post-mating behavioural changes, in terms of both traditional quantitative genetic and next-generation sequencing transcriptomic approaches. Our results include the discovery of rather limited male-input into female physiological changes post-mating (contrasting with Drosophila melanogaster for example), along with changes in olfaction- and venom-related gene expression. Oviposition also leaves a footprint in the transcriptome, providing clues as to the genes influencing sex allocation decisions. However, the limitations of such techniques will also be discussed, not least our limited understanding of the function of many insect genes.

Keywords:*Nasonia, Sex allocation, Polyandry, Receptivity, Transcriptomics, Sexual selection*

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Athalia rosae: An emerging genetic model system for sawflies

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Sawflies belong to the primitive Hymenoptera and are known to be pests of cultivated plants, flowers and forests. The turnip sawfly, Athalia rosae, is one of those species, a pest of cruciferous plants, and has unique biological features representing the order, such as parthenogenesis, haplo-diploidy and sex-linked occurrence of meiosis. We developed practical techniques to use this species as a model system: artificial manipulation of egg activation and fertilization, sperm cryopreservation, transposon-mediated germline transformation and RNAi in most developmental stages. Genome sequencing of A. rosae is ongoing and tools especially for analyzing gene function in vivo are prerequisite for utilizing these genomic data. It is thought that genes related to reproduction and development are possible candidates for biological control, for example, for applying to transgenesis-based sterile insect technology. We examined the function of the boule (bol) gene, which encodes RNA-binding protein functioning as a translational regulator, essential for meiosis in male animals. It is intriguing that bol homologues are conserved in the genome of Hymenoptera in which haploid males are devoid of meiosis in spermatogenesis. Some isoforms of the bol transcripts were found in the transcriptome. One was expressed exclusively in the testis and knockdown of bol by RNAi resulted in the absence of mature sperm without affecting any other phenotypes. Knockdown of the presumed downstream target gene, cdc25, showed a similar phenotype. The bol gene is required for progression of maturation division (but not meiosis) in haploid males and its defect leads to male sterility.

Keywords:*RNA interference, spermatogenesis, maturation division, male sterility*

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Genetics and genomics of fig wasp

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Figs and fig-pollinating wasps are obligate mutualists that have co-evolved for over 60 million years. During the long evolutionary history, the pollinators have evolved many characters adaptive to their lives within the unique compact syconia provided by the figs. We here try to trace these adaptive characters in the genome. We present a draft genome of Ceratosolen solmsi (Hymenoptera: Chacidoidea: Agaonidae) associated with Ficus hispida (Ficus: Moraceae), assembled from Illumina Sequencing reads obtained from all the male offspring of the same grandmother wasp. We used comparative genomic methods to obtain insight into the unique features of the C. solmsi genome. We found that this genome has much fewer genes annotated than its close relative Nasonia vitripennis. Additionally, we identified that C. solmsi harbors the least putative olfactory receptors genes except another insect species obligatory parasitic to human, the body louse. We also studied the genes composition of the immune systems, including the insecticide resistance and dietary detoxification. Putative horizontal transfer elements were also been screened. Some of these and other unique aspects of the fig pollinator genome are likely linked to the peculiar living environment within the compact syconia of this species.

Keywords:*obligate mutulism, nonmodel organism, de novo genome assembly, host specification.*

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Wratten. Introduction. Get rich or get even? Linking biodiversity and natural pest control

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Declining species number (richness) harms ecosystems, and conservation efforts have largely focused on conserving or restoring particular rare species. However, greater disparity in species relative abundances (evenness) might also do ecological harm, which could only be reversed by altering the densities of many species at once. We found that organic agriculture restores even relative-abundances among predator and pathogen biological control agents, dampening pest outbreaks and increasing plant growth. Thus, pesticide-reduction schemes such as organic agriculture provide a particularly promising avenue to restore functional evenness to ecosystems. The restoration of evenness is an achievable goal that can provide substantial ecological benefits.

Keywords:*species richness, evenness, predator, pathogen, organic farming*

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Flower power? Effects of apple orchard groundcover management on green apple aphids (*Aphis* spp.) their predators and on the canopy insect community

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Effects of habitat diversification through ground cover management on green apple aphids [Aphis spp. (Homoptera: Aphididae)], their insect natural enemies and the most abundant canopy insects (Neuroptera, Fulgomorpha, Cicadomorpha, Heteroptera, Coleoptera, Formicidae) was studied in an experimental apple orchard over six years. The abundance of woolly apple aphids [Eriosoma lanigerum (Homoptera: Aphididae)] was assessed in one year when their incidence was high. The habitat diversification was achieved by sowing annual and perennial flowering plants in the alleys of an apple orchard (FLOWER). Plots with weed free bare ground (BAREgr) and with regularly mowed grass (GRASS) served as controls. We found no evidence that flowering herbs sown in the alleys in comparison with grass ground cover or bare ground enhanced the biological control of green apple aphids. However, greater plant coverage in FLOWER enhanced the woolly apple aphid infestation. Increased plant coverage and diversity in FLOWER compared to BAREgr resulted in an increase in numbers of Chrysoperla carnea (Neuroptera, Chrysopidae) adults, leafhoppers and treehoppers, planthoppers, herbivorous (non-apple feeding) beetles, dipterans and parasitoid wasps in the canopy of apple trees. Abundances of these groups in GRASS in most cases were between those in FLOWER and BAREgr. Significantly greater species richness and diversity was found in FLOWER than in BAREgr for most of the studied functional groups although the number of predacious insect species was identical in all treatments.

Keywords:*Apple orchard, Cover cropping, Flowering herbs, Green apple aphids, Woolly apple aphid*

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The biology of *Holepyris hawaiiensis* (Hymenoptera: Bethylidae) parasitoid on *Ephestia cautella* (Lepidoptera: Pyralidae) under laboratory condition

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Abstract The Ephestia cautella is one of the major pests of palm grove and stored date in Iran. In this research, during 2008-2011 in Shahdad, Andijerd, and Kerman province (the south eastern of Iran) Holepyris hawaii is found in high potential in date storage and palm grove. Holepyris hawaii is an ectoparasitoid and gregarious wasp which was collected and reported for the first time in the Middle East. In this essay biology of Holepyris hawaii was studied on larvae of Ephestia cautella which were obtained from the IRIPP, where the culture had been intermittently reared on a semi artificial diet (Marouf et al., 1389) under laboratory conditions (26±2ºC, 65±5RH, and LD14:10). The research duration of the egg, larva, pupa, and the mean total development time for the adult of Holepyris hawaii were 3/07±0/093, 5/06±0/54, 8/13±0/57, and 18/32±0/11 days, respectively.

Keywords:*Ecto-parasitoid, Egg laying structure, Holepyris hawaii.*

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Seasonal patterns in the biodiversity of natural enemies in California walnut orchards

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There has been growing concern that the loss of biodiversity associated with intensification of agricultural production will lead to a reduction in the pest control services provided by the natural enemies of crop pests. It is often considered that natural enemy biodiversity is of greatest importance early in the cropping season in annual crops, but for perennial tree crops biological control services are often required throughout the cropping season. However, little is known of the seasonal pattern of biodiversity of natural enemy species in perennial crops and how this might influence the provisioning of pest control services for foliar pests that are active throughout the season. We monitored the seasonal biodiversity of natural enemies in California walnut orchards in 2010-2011 to determine patterns of change in the overall species richness and diversity of foliar natural enemy species through use of herbivore-induced plant volatiles baited sticky panel traps. We present the seasonal patterns observed for general insect predators and parasitoids, and discuss how these patterns and the observed variation between years and locations are likely to influence the pest control services provided by natural enemies in this important tree crop.

Keywords:*Predator, parasitoid, trap, HIPV,*

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Insect Biological Control

Disentangling the spider's web

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Agroecosystems, although subjected to monocultural practices that typically suppress arthropod diversity, are surprisingly rich in invertebrate fauna. Temperate agricultural production systems, for example, contain at least 1,500-3,000 species and thus these rather homogeneous crop environments offer resources to arthropod communities that not only allow survival, but foster growth and development of individual populations. Furthermore, the predisposition of many predators to remain in place despite limited resource availability, driven by the inherent risks associated with site or habitat abandonment, facilitates their capacity for pest suppression, potentially impacting prey populations during colonisation. Spiders, dominant predators within such habitats, exhibit many favourable attributes that make them valuable biological control agents. Indeed, their diverse foraging habits, rapid colonisation ability, high levels of site tenacity and, in many cases, voracious appetite for pest species should place them at the forefront of many conservation biological control schemes. This paper will use multi-disciplinary approaches to field ecology to disentangle the complex spider food web in agroecosystems, characterizing the functional role of spiders and examine how in-field diversity impacts biological control services.

Keywords:*biodiversity, molecular ecology, predator-prey interactions, Araneae*

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Biological control in Kentucky winter wheat: Using molecular tools to assess sustainable management techniques

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Aphids are pests of multiple cropping systems, due to the viruses they vector and their rapid rate of population growth. In Kentucky winter wheat, grain aphids (Rhopalosiphum padi, Sitobion avenae) cause substantial yield loss as vectors of Barley Yellow Dwarf virus (BYDV), prompting routine insecticide applications to be applied. Importantly, these aphids are preyed upon by foliage dwelling predators and, when they fall to the ground, epigeal spiders which play a major role in preventing re-colonization of the plant. Consequently, natural weed strips were created to increase natural enemy abundance in the wheat, with the hypothesis that they will promote natural suppression of pestiferous aphid populations. In a multi-faceted approach using spatial, temporal and molecular analysis of predation, this hypothesis was tested with extensive collection of plants, aphids and natural enemies. In the laboratory, samples were indentified and screened for BYDV using enzyme linked immunosorbent assay to quantify the level of infection in the field. Additionally, species richness and diversity counts were conducted to examine the effect that natural weed strips may have on the existing arthropod populations. Results will be discussed in the context of conservation biological control, and how low-cost, low-input manipulations may impact viruliferous aphid populations.

Keywords:*Aphididae, conservation biological control, habitat manipulation*

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Improved fitness of the parasitoid *Aphytis melinus* receiving sugar subsidies in the field

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Many adult parasitoids depend on sugar-rich foods such as nectar and honeydew to meet their energy requirements. Here, we first use HPLC (high performance liquid chromatograph) analyses to assess the sugar reserves (total sugar per parasitoid) and honeydew consumption of individual field collected Aphytis melinus parasitoids, the most successful biological control agent of California red scale Aonidiella aurantii. The number of honeydew producers was also measured when A. melinus were collected. Our data show that A. melinus, whose host does not produce honeydew, fed commonly on honeydew from other abundant hemipterans in spring and summer. However, when the number of honeydew producers decreased, as occurred in fall, less than 20% of A. melinus were found to have fed on honeydew. In fall, the average total sugar content in A. melinus was reduced three-fold. In a second assay, we determine whether the addition of sugar subsidies might improve the fitness and performance of A. melinus when honeydew producers were scarce. For this, we compared the sugar reserves of A. melinus using the HPLC analysis, its population density, parasitism rates on A. aurantii and migration in trees with and without sugar subsidies. Our result show that the number of adult A. melinus per tree, migration, parasitism rates and the number of parasitoids classified as sugar fed (those that had previously fed on sugars) was higher in trees were sugars were added.

Keywords:*nutritional ecology, hymenoptera, carbohydrate, honeydew, HPLC*

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Potential predators that may contribute to pests control in future irrigated areas around the newly constructed Hamdab Dam in Northern Sudan

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The Hamdab (Merowe) dam, which has been constructed recently on the Nile River is located in Northern State, an area lies within the desert arid zone of the Sudan. However, this area is climatically suitable for growing several field and horticultural crops. Therefore, the dam was expected to irrigate thousands of hectares of arable virgin lands along the Nile in the near future. Regrettably, very little research has yet been carried out in that part of the country regarding agricultural pests and their natural enemies. In this study, regular surveys continued for one year (2010/2011) were carried out at three different locations near the dam, where the prevalent predatory species of agricultural pests were reported and counted on certain crops. Hence, the population densities of important predators were compared among the different crops and seasons. Since chemical insecticides are very rarely practiced in the Northern State, numerous species of natural enemies were found flourishing on the studied crops, including mainly, chrysopids, coccinellids, syrphids and spiders (e.g., thomisids, salticids and cob weavers). Consequently, the promising predators reflected the highest prevalence and impacts on major pests were indicated. These predators should receive great attention, as potent natural control agents, whenever agricultural expansion is realized.

Keywords:*Predators, seasonal abundance, Hamdab dam, Northern State, Sudan.*

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Biodiversity and biocontrol: Putting it all together in Asian rice

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This presentation will provide an overview to laboratory and field results from a three-year, multi-country assessment of the efficacy of ecological engineering for enhancing biological control of rice planthopper pests. Laboratory studies focussed on the response of key parasitoids Anagrus nilaparvatae and A. optabilis and the important predatory mirid Cyrtorhinus lividipennis to potential nectar plants for use on the bunds around rice fields. Y-tube olfactometer studies with sesame, Impatiens balsamena, Emilia sonchifolia, Tagetes erecta and Emilia sonchifolia indicated that sesame most consistently proved attractive to adult natural enemies, maximised longevity, enhanced parasitism or predation of brown planthopper eggs and reduced handling time. Sesame also had the practical advantages of being long-flowering and valued by farmers as a secondary crop. Field experiments in China, Vietnam and Thailand showed that using locally appropriate nectar plants (commonly sesame) on bunds led to natural enemy numbers and diversity being markedly enhanced and good control of rice planthoppers under conditions of greatly reduced insecticide inputs. Rice grain yields were maintained and economic analysis suggest this ecological engineering approach to be favourable to conventional farmer practice. Furthermore, frog numbers were increased, suggesting a wider benefit to biodiversity. Up-scaling of ecological engineering for rice is now underway in each country, funded by significant funding by the respective Governments for promotional materials and demonstration sites.

Keywords:*parasitoid, predaror, mirid, nectar, sesame, ecological engineering*

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Niche engineering reveals complementary resource use among predators

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Greater resource use by diverse communities might result from species occupying complementary niches. Defining niches can be challenging, however, due to the difficulty in relating differences between species in particular traits to their use of complementary resources. Here, we overcame this obstacle by exploiting plastic foraging behavior in a community of predatory insects. These predators complemented one another by partitioning foraging space, with some species foraging primarily along leaf edges and others at leaf centers. Indeed, on intact leaves, edge- and center-foraging predators combined to kill more prey than any single predator species could by itself. These emergent diversity effects, however, disappeared on plants damaged by the caterpillar Plutella xylostella. Caterpillar chew-holes brought edge habitats to the center of leaves, such that all predator species could attack aphids anywhere on the plant. With spatial niche differences diminished, there were no benefits of predator diversity; the most voracious single predator species killed the most aphids. Thus, caterpillar herbivory determined whether multi-predator-species effects reflected complementarity or species' individual impacts. Our study provides direct evidence for a causative relationship between niche differentiation and increased resource consumption by diverse communities, as revealed by ecological engineers that homogenize the foraging environment.

Keywords:*biodiversity, evenness, predators, pathogens, organic agriculture, pest control*

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Agricultural intensification drives landscape effects on host-parasitoid interactions in agroecosystems

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Agricultural land use threatens biological control by natural enemies because of simplification of habitat structure and intensification of disturbance and agrochemical inputs. Here, we disentangle the mechanisms driving landscape-scale effects on host-parasitoid interactions by taking advantage of a landscape modification gradient in which the diversity of habitat types and annual crop cover in the landscape were uncorrelated. We quantified herbivore densities and parasitism and hyperparasitism rates on two important crop pests across 30 landscapes. We used structural equation modelling (SEM) to test whether land-use intensity (insecticide application and habitat disturbance) or resource availability for parasitoids (floral resources and alternative host plants) were mediating the effects of habitat diversity and annual crop cover in the landscape. Rates of primary- and hyper-parasitism of aphids and primary parasitism of P. xylostella decreased with increasing annual crop cover, whereas decreasing habitat diversity in the landscape had little effect on parasitism rates. These effects were mediated almost entirely by greater habitat disturbance and greater frequency of insecticide application, rather than by changes in resource availability. Our work suggests that increased land-use intensity (e.g. higher insecticide inputs and greater levels of disturbance associated with increasing area of annual crops) has been underestimated as a driver of landscape effects on host-parasitoid interactions. These findings have important implications for the maintenance of biological control. The promotion of low-intensity farming practices that limit the extent and frequency of agrochemical inputs and habitat disturbances will be essential for the maintenance of effective biological control by parasitoids in agroecosystems.

Keywords:*diamondback moth, disturbance, ecosystem service, grey cabbage aphid, habitat type diversity*

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Agricultural policy-induced landscape changes: Effects on carabid abundance and their biocontrol potential

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Biodiversity plays a key role in management of most agroecosystems, and potentially can affect biological control of insect pests. However, diversity of crop food webs can be negatively affected by the recent crop management practices, including use of synthetic herbicides and/or artificial fertilizers (especially nitrogen), which can alter abiotic and biotic components of the agroecosystems. We studied the relative importance of (1) nitrogen input, (2) pesticide use and (3) environmental factors (field size, landscape diversity, neighbouring cultures and habitats, altitude) on ground beetle community structure, diversity (Shannon-Wiener index) and abundance in 150 wheat fields or parts thereof, which belong to 30 farms over a period of 2 years, using pitfall trapping. In each year, two collections were made, one in spring and one in summer. This enabled us to include seasonal and annual variations. The results are of strong practical importance, as ground beetles are supposed to have a potential role in suppression of cereal aphids; the latter were reported to be responsible for up to 15% decrease in yield and aphid outbreaks are related with agricultural intensification.

Keywords:*community structure, species richness, Shannon-Wiener index, agricultural management*

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Economic impact of termite pests and current control practices

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Drywood and subterranean termites are the number one structural pest in the US with an economic impact of nearly $ 3 billion annually. Subterranean termites represent about 75% of this figure and their impact is more widely distributed than drywood termites. The primary incidence and major damage caused by drywood termites being restricted to Arizona, California, Florida, and Texas. However, the urbanization of the desert southwest has resulted in increased termite activity. Invasive species pose a special problem for the Gulf States and Florida. The two primary strategies for controlling drywood termites are entire structure treatments and localized treatments. Fumigation or heat treatments are the two viable options for treating the entire structure. Of the various localized treatments, only the localized chemical treatments have shown much promise for remedial control. Injections of spinosad, fipronil, thiamethoxam, and boric acid dust into galleries have provided excellent control and are promising as localized treatments. For nearly 60 years, the principle method of remedial control of subterranean termites has been the application of pesticides to soil to provide a chemical barrier. In the last decade, soil toxicants with delayed toxicity and low repellency have replaced the classic repellent chemistries such as the pyrethroids for remedial control. With the advent of dose independent chitin synthesis inhibitors such as hexaflumuron, noviflumuron, and diflubenzuron, baiting technology has dramatically expanded especially for subterranean species that are aggressive foragers.

Keywords:*drywood termites, subterranean termites, insecticide barriers, baits*

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Termite pest species and their control options in Japan

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Of 23 termite species distributed in Japan, only 5 species are recognized as pests. They are Reticulitermes speratus, R. kanmoensis, Coptotermes formosanus, Cryptotermes domesticus and Incisitermes minor: three subterranean and two dry-wood species. At present, more than 99% of termite damage in Japan is attributed to subterranean species, however, dry-wood termite damage, particularly by I. minor, is now increasing. These days the termite control market in Japan is estimated as 70 billion JPY (= 900 million USD). Similar to the other countries with subterranean termite problems, soil/timber treatments by liquid formulations with non-repellent active ingredients are the most popular method. In Japan, the soil treatment is basically conducted inside the foundations, and the perimeter treatment is not common due to environmental concerns. Construction timbers up to one meter from the top of the foundation are normally treated by brushing/spraying. Concern about the leaching-out from the treated timbers restricts the use of borates. Pressure-treated sills are commonly used in residential houses. The testing methods of chemicals against termites are standardized by the JIS K-1571(2010). Baiting and the installation of physical barriers are not yet widely used in Japan, mainly due to their costs. Governmental policy for promoting the construction of long service-life buildings, for both, residential houses and also public buildings, is now enhancing the development of technologies for inspection/diagnostic systems of termite infestations. Since fumigation of buildings is difficult in Japan, finding novel control systems against I. minor will be the big issue in the near future.

Keywords:*termite control, long service-life buildings, pressure-treated sills, on-site treatment, JIS K-1571*

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S1201M03

Urban, Stored Product and Post Harvest Entomology

Termite pests and their management in Southeast Asia

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Termites are an important group of urban insect pests in South East Asia. In Malaysia, Coptotermes gestroi accounted more than 85% of the total damages of buildings and structures, and similar observations were recorded in Thailand, Indonesia and the Philippines. It is also common to find several termite pest species co-existing and infesting buildings and structures. In Malaysia, other species that can be readily found in- and around buildings and structures, including C. curvignathus, C. kalshoveni, Microcerotermes crassus, Macrotermes gilvus, Macrotermes carbonarius, Odontotermes spp., Schedorhinotermes spp and Microtermes pakistanicus. For drywood termites, Cryptotermes cynocephalus is the common species in Malaysia and Indonesia. In Thailand, Microcerotermes crassus is gradually becoming an important pest species in buildings and structures built on former rice field in suburbia. In the Philippines, M. gilvus, Microcerotermes losbanosensis and Nasutitermes luzonicus were most prevalent after C. gestroi. For drywood termite, Cryptotermes thailandis occasionally spotted in wooden houses and furnitures mostly in the southern part of the country both inland and along the coastline or on the islands. In the Philippines, the most important drywood termite species is Cryptotermes dudleyi. In South East Asia, it has been observed that baiting activity has increased the prevalence of secondary species in buildings and structures.

Keywords:*Termites, Southeast Asia, management, baiting, secondary pest*

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S1201M04

Urban, Stored Product and Post Harvest Entomology

Termite control practices and recent IPM project in mainland of China

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There are about 10 species of harmful termites in China. Most of them distribute in the south of China. BC 220 years , termite control was recorded in some classical works. But before 1950, termite control area was very small and the means rarely . During recent decades, great progress has been made whether in the control area or the control theory and technology. Especially, since July 2006 , an IPM demonstration project entitled "Demonstration of Alternatives to Chlordane and Mirex in Termite Control" by GEF fund supporting, WB and Chinese government participating has been carried out under the frame of the Stockholm Convention on Persistent Organic Pollutants (POPs). By December 30, 2011, three demonstration provinces have installed 694,100 in-ground(IG) and 151,500 above-ground(AG) bait systems with preventive treatment covering 77.2372 million square meters and remedial treatment 3.805 million square meters, relating to residential buildings and all types of architecture for purposes of culture, public health and historic heritage. A significant effect of control has been shown after use of the monitor-controlling system. By the use of this system ,the termite threat has been eliminated from those demonstration items and the use of chemical insecticide has been reduced greatly.Through this project, we have completely eliminated the use of chlordane and Mirex in demonstration provinces and got a lot of useful experience not only in management and technology but also in understanding IPM theory on termite control in China.

Keywords:*termite control practice, China, IPM, demonstration project, monitor-controlling system*

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Termite pests and their control in Taiwan

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Five of the 17 recorded termite species in Taiwan are considered as pests of wooden structures and wood products. Two subterranean termites, Coptotermes formosanus Shiraki and Coptotermes gestroi (Wasmann), are the major pests and responsible for > 87% of termite infestations in urban area. Both species are most found at low land area < 500 m. C. formosanus is distributed throughout Taiwan, but C. gestroi is restricted in southwest. Another subterranean termite, Reticulitermes flaviceps (Oshima), occasionally attacked manmade structures, resulting in < 1% infestations. R. flaviceps are commonly found in mountainous areas and lowland of northern Taiwan. The drywood termite, Cryptotermes domesticus (Haviland), is responsible for 6% infestation mainly in southern Taiwan. The fungus growing termite, Odontotermes formosanus (Shiraki), is the most common termite species in Taiwan distributed from lowland up to 1,200 m altitude. O. formosanus is a minor urban pest contributed 6% structural infestation but a major agricultural and forestry pest. Currently, 116 pesticide products are labeled for termite control in Taiwan and nine of them were specialized for this purpose. Eight active ingredients including boric acid, hexaflumuron, noviflumuron, fipronil, imidacloprid, bifenthrin, cypermethrin, and permethrin are used in the nine specialized termiticide products. Organophosphates and pyrethroids are used primarily in the non-termite-specific pesticides. Based on the questionnaire survey in 2009, the liquid pesticide with fipronil as the active ingredient was used in 75% of all cases and termite bait containing hexaflumuron was employed in 20%.

Keywords:*Coptotermes formosanus, Coptotermes gestroi, liquid termiticide, termite bait*

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S1201M06

Urban, Stored Product and Post Harvest Entomology

Non-repellent termiticides: Development, key features, efficacy and control

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In past decade, non-repellent termiticides have emerged as a popular treatment alternative in post-construction infestations. Most of these termiticides have performed very well in the field compared to the repellent termiticides used in the past. The superior performance of these new generation termiticides is attributed to their non-repellency, delayed toxicity and their ability to be transferred from exposed to unexposed worker termites. However, researchers have demonstrated that several factors such as dose, period of exposure, and mode of action of the insecticides also play a significant role in the efficacy and horizontal transfer of non-repellent termiticides. In addition, all the termiticides are applied to the soils and therefore soil physical and chemical characteristics also have an effect on the bioavailability of these termiticdes to the termites tunneling in treated zones. Soil types may also affect degradation and movement of these termiticides in the soil. Thus, the efficacy and performance of these termiticides should be studied in relation to all the above mentioned factors to know their key attributes.

Keywords:*Delayed Toxicity, Non-repellency, Horizontal Tansfer*

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S1201M07

Urban, Stored Product and Post Harvest Entomology

Development of baiting as a method to control subterranean termites

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As early as the 1960's a "bait method of control" for subterranean termites was discussed in scientific literature. The concept described at the time was to use toxicant-impregnated wood blocks to suppress populations of pest termite species in the field. In the years since then, considerable research has been conducted to investigate potential active ingredients for termite bait, including pathogenic fungi, metabolic inhibitors such as sulfluramid, chitin-synthesis inhibitors such as diflubenzuron, lufenuron, and hexaflumuron, and even fipronil and encapsulated permethrin. Additional work has been published on associated stations, matrices, sensors and attractants. Focused research efforts resulted in the first termite baiting system being commercially introduced in 1995. Termite baiting systems now represent a substantial portion of the termite control market in the USA and a growing proportion of the termite control market globally, with research continuing to refine many of the elements earlier defined.

Keywords:*subterranean termite, baiting, control, hexaflumuron*

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S1201M08

Urban, Stored Product and Post Harvest Entomology

IPM area-wide projects and population dynamics of subterranean termites

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With the availability of commercial baits capable for eliminating colonies of subterranean termites, several area-wide projects have been attempted. Most of these projects follow the conceptual framework of termite IPM (Integrated Pest Management) as suggested by Su & Scheffrahn (1998), i.e., to manage termite populations via colony elimination by using less persistent and less toxic insecticides at smaller quantities. Area-wide projects in the US and the termite IPM project implemented in three provinces in China will be discussed. One vital issue for area-wide projects is the recovery process of subterranean termite populations in target areas following the total elimination of all detectable colonies. In the 34-acre Armstrong Park of New Orleans, Louisiana, activity of the Formosan subterranean termite reached a full recovery 5-6 years after all detectable colonies had been eliminated. The Park was repopulated by three types of colonies, i) small colonies that were undetected during the initial elimination attempt (concealed colonies), ii) incipient colonies initiated by alates flew in after the total elimination, and iii) established colonies neighboring the Park.

Keywords:*Isoptera, Rhinotermitidae, Coptotermes, Reticulitermes, termite baits, colony elimination*

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Urban, Stored Product and Post Harvest Entomology

A simulation study on the territorial competition of subterranean termites

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The foraging territories of 2 subterranean termites, Coptotermes formosanus Shiraki and Reticulitermes flavipes (Kollar), were simulated using a two-dimensional model to explore how territorial competition changes with 4 variables characterizing the formation of territory: the number of primary tunnels, N_0; the branching probability, P_branch; the number of territories, N; and the blocking probability, P_block. The blocking probability P_block quantitatively describes the likelihood that a tunnel will be terminated when another tunnel is encountered; higher P_block values indicate more likely termination. We defined a territory as a convex polygon containing a tunnel pattern and explored the effects of competition among termite colonies on territory size distribution at steady state attained after sufficient simulation time. At the beginning of the simulation, N = 10, 20, …, 100 initial territory seeds were randomly distributed within a square area. In our previous study, we introduced an interference coefficient γ to characterize territorial competition. Higher γ values imply higher limitations on network growth. We theoretically derived γ as a function of P_block and N. In the present study, we considered the constants in γ as functions of N_0 and P_branch so as to quantitatively examine the effect of tunnel structure on territorial competition. By applying statistical regression to the simulation data, we determined the generalized γ functions for both species. Under competitive conditions, territory size is most strongly affected by N_0, while territorial competition is most strongly affected by N, followed by P_block and N_0.

Keywords:*Termite territorial competition, Termite tunnel network, Termite simulation model*

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Community-based termite baiting projects in California

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Community-based termite baiting projects in California. Vernard R. Lewis University of California, Berkeley Department of Environmental Science, Policy and Management137 Mulford Hall #3114 Berkeley, CA 94720-3114. Abstract ? I will review several community-based termite baiting projects for Northern and Southern California. All studies involved species of subterranean termites found in California, primarily the Western subterranean termite, Reticulitermes spp. The study areas were considerable in size, ranging from several to more than 36 ha. For the Northern California site, 550 in-ground bait stations were installed and monitored around thirty-three buildings. The Southern California was considerably larger; over 7,300 in-ground bait stations were installed and monitored around 140 structures. Discovery time and percentage of stations hit for in-ground stations by foraging termites was typical for California's Mediterranean climate, several months and 12% during the first year. However, the use of ancillary stations, applications of sugar water, and control service visits to monitor for termite foraging and feeding achieved higher station hit rates and bait consumption. The disappearance of termites from stations and the drastic reduction of consumer complaints were consistent and supportive of colony elimination for many colonies at and surrounding both sites. The use of area-wide management practices that includes monitoring and baiting is a strategy that is consistent with Integrated Pest Management principles, while also providing the framework and mechanism for termite remedial control and prevention. Lastly, significant savings to the environment can be realized by lessening the need for high volume applications of liquid termiticides

Keywords:*Area-wide management, community-based projects, subterranean termites, foraging, baiting*

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Community-based subterranean termite projects in New Orleans, Louisiana.

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The Formosan subterranean termite (FST), Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae) is a destructive, introduced pest species indigenous to Southeast China. This termite has become established in New Orleans and it has supplanted native subterranean termites as the primary structural pest species of structures and trees. In 1998, an area-wide termite project was initiated to reduce the numbers of FST in the French Quarter. This project was the first and largest area-wide termite management program of its kind. This program has saved the French Quarter including its historic structures and trees from devastation by the Formosan subterranean termite. Among the project's many goals was obtaining and utilizing basic FST biological information to maximize control efforts. In this vein, the program has developed novel termite detection tools which are now used by the pest control industry. Public and industry education was a major component of the project. People continue to unwittingly transport this devastating termite to new areas each year. Public awareness and education is the first step to stopping the spread of this invasive species. Operation FullStop technologies, techniques, and educational materials are being utilized nationwide by the pest control industry. Operation FullStop was a unique and successful partnership among the federal government, local government, universities, private industry, and non-profits resulting in great economic benefit for the general public.

Keywords:*Formosan subterranean termite*

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From research to end user: Community-based termite management in Chile

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Area wide termite management research in Santiago, Chile began in 1999. Studies to show the effect of Hexaflumuron termite bait were carried out in a 5 hectare residencial setting in the community of Cerro Navia. These studies verified the effectiveness of the Sentricon baiting system for the management of Reticulitermes flavipes foraging populations. The study showed that intense baiting in a limited number of bait station clusters eliminated pest activity at distances up to 60 meters. These findings lead to the development of a low cost area-wide protocol for baiting subterranean termite populations, especially for use in low income settings. After three years of field research, termite elimination projects were carried out in various municipalities within Santiago and Chile´s fifth region. Initial field baiting was performed by trained pest management professionals. These same professionals trained municipality staff in the biology, behavior and management of subterranean termites. The baiting system was then made available to the municipalities for use by their own trained employees, within their integrated pest management programs. These efforts have been extremely cost effective, have eliminated termite foraging populations and the use of toxic chemical barriers.

Keywords:*reticulitermes flavipes, area wide baiting*

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S1201M13

Urban, Stored Product and Post Harvest Entomology

DNA genotyping for assessment of treatment success in area-wide termite control

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The cryptic life style of subterranean termites makes direct observation of colony numbers, sources of alates and treatment success difficult. Microsatellite genotyping, i.e., the analysis of repetitive sequences in the genome that are variable in length and are inherited in a Mendelian fashion, provides a method to characterize termite colonies and assess treatment success on the colony and population level. This talk presents how DNA profiles were used in several model studies of the Formosan subterranean termite (Coptotermes formosanus) in New Orleans, Louisiana, USA. By assigning termites to colonies via their DNA profiles much needed information was gained about size and location of foraging areas of termite colonies, colony numbers, colony movement, and breeding system of colonies. Using DNA profiles it was determined that the treatment program truly eliminated all treated colonies regardless of the size of their foraging area and their breeding system. Since New Orleans has high termite pressure, termites re-infested the study area again after treatment. DNA profiles revealed that the re-infesting termites did not originate from the previously treated colonies, i.e. treatment worked but other colonies move into vacated areas. Light-trapping of alates and relatedness calculations were used to estimate the numbers of colonies contributing alates to termite swarms and to monitor the decline in the number of mature colonies in the area-wide treatment program in New Orleans.

Keywords:*microsatellite genotyping, colony assignment, alate, infestation source*

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The mechanics of tunnel excavation in subterannean termites, a preadaptation to fungal gardening.

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Since Grasse's groundbreaking work (1959), construction and excavation in termites and ants has been thought to be coordinated through a system of threshold responses to pheromones. Recent studies on subterranean termites suggest pheromones may play no direct role in the removal of soil, relegating their function to recruitment. In this new model, tunnels are viewed as extensions of the termite phenotype and serve as an organizing factor, limiting movement to specific vectors, and providing unambiguous starting and ending points. Tactile information and traffic density provide the template coordinating the labor of excavators. When tunnels are too narrow for the number of termites attempting to gain access to soil at the tunnel's end, a queue forms and termites take soil from the tunnel walls wherever they are held up. Widening of the tunnel end can lead to bifurcation, while depressions in tunnel walls left by periodic widening of tunnels act as cues for future branching. Excavated soil is deposited along the walls and inside depressions, acting to narrow tunnels and inhibit branching. At food sources, termites transition from excavating through soil to excavating into wood. The tailings, small wood fragments, are deposited along tunnels in the same manner as soil. The result is that tunnels near food sources are lined with wood fragments. This sequestered wood, subsequently covered in a fecal envelope, is a unique microhabitat, and may be the route of fungal invasion that was a necessary precursor to the type of fungal gardening seen in more derived termite species.

Keywords:*Termite Tunnel fungus garden self organization excavation*

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Termite movement efficiency in their tunnel galleries

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Subterranean termite, Reticulitermes speratus kyushuensis (Isoptera: Rhinotermitidae), excavate complex tunnel networks below the ground for foraging. The tunnels are either curved or meandering. In the present study, we explored how the time spent inside a tunnel changes in accordance with the degree of tunnel curvature. To do so, we constructed artificial tunnels with different curvatures in acryl substrates. The tunnel was 5 cm in length with widths of W (W = 2, 3, or 4 mm), and the distance between the 2 ends of the tunnel was D (D = 2, 3, 4, or 5 cm). A higher value of D means a lower curvature. We measured the time (τ) taken by a termite to pass through the tunnel. In the case of W = 2 mm, the values of τ were statistically equal for D = 2, 3, or 4 cm, while that for D = 5 cm was significantly lesser. In the case of W = 3, τ was statistically more for D = 2 and 3 cm than it was for D = 4 and 5 cm. For W = 4, τ was statistically equal for D = 2 and 3 cm, while τ for D = 4 cm was relatively shorter. Interestingly, the value of τ when D = 5 cm was statistically the same when D = 3 or 4 cm. These resulted from 2 types of termite behavior, biased walking and zigzag walking.

Keywords:*Termite tunnel curvature, foraging efficiency, termite tunnel network*

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S1202TU01

Urban, Stored Product and Post Harvest Entomology

Pest of stores and stored products-an archaoentomological perspective

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The history of pests of stored products provides insights into the origins of storage and agriculture as well as trade and changes in economy. The flightless grain weevil, Sitophilus granarius spread from the Fertile Crescent with the origins of agriculture to the eastern Mediterranean and into Central Europe. Later, it was carried to the northern margins of the Roman Empire and beyond with the Vikings to the North Atlantic islands. It provides an example of the biogeographic implications of introductions of pests and their impact on early agriculture and trade. Palaeoentomological work from archaeological sites in the Eastern Mediterranean allows some understanding of processes involved and information on the early dispersal of storage pests, which included Rhyzopertha dominica, Tribolium castaneum, and Lasioderma serricorne among others. Potentially these early Neolithic and later sites will chart the progress of introductions and routes of dispersal. The process of introductions of pests in the North Atlantic, in particular to areas where there was no cereal cultivation is interweaved with European colonization of new lands and Hanseatic trade and provides some understanding behind the processes of species becoming cosmopolitan. From Northern Europe to the North Atlantic Islands and Canada this is a story of overcoming biogeographic barriers, accidental introductions and some improbable immigrants which accompanied people on most of their journeys.

Keywords:*archaeoentomology*

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Evolution of grain pests

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Employing a new examination method "impression method" which is the way to recover the living things from prehistoric potsherds have been giving us a lot of evidences on the history of plant utilization in Japan since 2005. One of the great discoveries deserves our attention is maize weevil, Sitophilus zeamais Mots.. In recent Japan we have two Sitophilus species which are S. oryzae L. and S. zeamais which are harmful insects infesting stored grains, mainly rice. Before our new discovery at Sanbonmatsu Site it had been believed that two species may have been diffused by the spread of rice agriculture in East Asia and have intruded into Japan accompanied with rice cultivation from Korea. However the Sanbonmatsu maize weevils dated back to ca. 10500 cal BP. In that time there was neither domesticated rice nor wild one in Japan. Therefore we estimated that maize weevil might be indigenous insect living in Japan since over 10000 years ago and has infested the stored dry seeds in Jomon villages, for example, acorn, chestnut, or legume and attributed it the world' oldest house pest. This suggests we must reconsider previous scenarios for the evolution and propagation of grain pest weevils, especially in eastern Asia. In order to study the problem we must seek the archeological evidence for Sitophilus species in Korea and China in further study. We believe future interdisciplinary collaborations among geneticists, entomologists, and archaeologists in the East Asia will solve the problem.

Keywords:*maize weevil, Archaeology, Neolithic, impression method, stored seeds*

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Invasive and synanthropic - updated aspects on the natural and cultural history of the webbing clothes moth *Tineola bisselliella* (Lepidoptera: Tineidae)

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It is generally accepted that the natural habitats of most pest insects can be found outside the synanthropic environment in layers of leaf litter, under bark, as well as in rodent or bird nests. Indeed, most of the common pests have been reported as being facultative nidicolous. Therefore infestation of commodities by pest insects out of these reservoirs is one considerable possibility. However, the likelihood of a pest´s occurrence and survival out-doors largely depends on its ecological potential and competitiveness against other species of the same ecological guild. Some pest species are rarely found in wild habitats, especially in those regions where they are not native and where they have been introduced by man. The fabric pest Tineola bisselliella serves as a good example. Most likely originating in Southern Africa this insect was introduced into Europe probably not earlier than the late 18th century. Being more tolerant to dry environments than other fabric pests its economical importance increased during the 20th century when in-door climates changed because of central heating systems. Its occurrence in out-door natural habitats must be regarded as accidental. Reported founds of webbing clothes moth larvae in bird nests e.g. have been largely overstated in the literature. This paper will try to reconstruct the natural and cultural history of T. bisselliella by summarizing autecological, behavioral, and archeological data in combination with critically analyzed published faunistic records. It will be argued that the webbing clothes moth is a true synanthropic species in most parts of the world.

Keywords:*pest insect, evolution, ecology, nidicolous*

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Urban, Stored Product and Post Harvest Entomology

The practicality of using molecular techniques for identification of stored-product insect pests

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Proper identification of species is significant for insect pests monitoring and management, quality control of stored products, and quarantine of imported grains. Molecular techniques based on DNA analysis are regarded as the rapid, viable, and economical methods of species identification which overcoming the non-adult and non-complete sample of individual insect and the lack of available taxonomic expertise. In recent years, different molecular techniques were employed to characterize and identify the species of stored insect pests, including DNA polymorphism assays, DNA barcoding, and species-specific primer. In this review, we summarized the studies and applications of molecular techniques to identify the common stored product pests. DNA polymorphism assays, such as RAPD, RFLP and DNA fingerprinting technique were widely used in rice weevil, maize weevil, saw-toothed grain beetle and psocids. DNA barcoding which based on the sequence of mitochondrial 16S rDNA, COI, and nuclear ITS rDNA was studied especially for stored psocids identification. Species specific primer made the stored-product insect pests identification more timesaving and inexpensive, e.g., the multiplex species specific PCR was developed to identify several psocid species, and the real-time PCR served as a rapid and accurate detection method of granary weevil and Indian meal moths. For the molecular identification of stored-product insect pests in the future, more international collaborations are expected.

Keywords:*stored-product insect pests, molecular identification, DNA polymorphism assays, DNA Barcoding, specific primer*

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Use of molecular methods for species diagnostics and to identify dispersal patterns of stored-product insects

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For the past decade my research group have used a range of nuclear and mitochondrial molecular markers to investigate the genetic diversity and global dispersal patterns of stored product pests e.g. booklice Liposcelis bostrychophila and L. decolor and the warehouse beetle, Trogoderma variable and agricultural pests e.g. western corn rootworm Diabrotica virgifera virgifera and the apple codling moth Cydia pomonella. Although a range of molecular markers have been used to study these species, similar genetic patterns between the species investigated have been found. In general for these pests the genetic diversity and structure is lower in their invaded range compared to their native range despite gene flow or dispersal being unrestricted and multidirectional. Genetic diversity is usually lower in introduced populations; however, diversity loss is mitigated by multiple introduction events that ultimately strengthen the diversity of the invaded populations. This has significant ramifications for control programs especially if resistant alleles are introduced into areas where they were not previously found. In this presentation I will present four short case studies of the invasive insects that I have studied to highlight how we have come to understand their invasion genetics through the use of molecular markers. I will discuss how global commodity trading has facilitated multiple introductions of pest insects and removed barriers to their dispersal. Also I will discuss which molecular markers are helping to inform quarantine diagnostics and integrated pest management practises.

Keywords:*molecular markers, IPM, stored product insects, microsatellites, invasion genetics*

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Recent advances of stored-product psocid research in China

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In the last two decades, psocids (Psocoptera) have become a serious problem in grain storages in China. To date, 1,588 species in 166 genera and 27 families and 3 suborders of psocids are known, whereas, only 27 species were described in the genus Liposcelis from China. Numerous paperss on psocid bionomics, physiology and biochemistry, control measures, and molecular biology have been published. The main differences about biochemical and toxicological characteristics of detoxification, target enzymes and energy sources were clarified among some psocid species. Some detoxifying enzymes (GSTs) and target enzymes (AChE) were purified in psocids and the biochemical and toxicological characterizations of these purified enzymes from different resistant strains and field populations were systematically analyzed. At molecular levels, the research mainly includes molecular identification of similar species, resistance mechanisms, population genetic structure, and detection of symbiotic bacteria. Gene cloning of detoxification and target enzymes and further mRNA expression level and heterogeneous expression of these functional genes were carried out. A detailed understanding of psocid gene flow patterns and the resultant genetic structure among populations within China and all previous researches were useful to understand the formation of resistance mechanisms of psocids to insecticides. These results not only fastened the acquaintance of psocid molecular toxicology and enriched the content of evolution and genetic variation, but also contributed to development of a molecular diagnostic technique for psocid resistance in the field and paved the way for designing new insecticides and developing new strategies for pest management.

Keywords:*Psocids, resistance, mechanisms, genetic strcuture, management*

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Recent advances in development of attractants for stored-product insects

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Pheromones and other attractants are widely sued for IPM stored-product pests, mainly through use of traps to detect and estimate insect numbers for management decision-making. Pheromone-baited monitoring traps are routinely used for stored-product moths such as Plodia interpunctella and Cadra cautella, the cigarette beetle, the warehouse beetle and the Tribolium flour beetles. A recent breakthrough in pheromone-based suppression was the registration of a common moth pheromone for mating disruption if Indianmeal moth and its relatives. Mating disruption shows promise for control of the cigarette beetle. The male-produced aggregation pheromone system of Tribolium castaeum was recently elucidated further with regard to stereochemistry of the chiral pheromone, and this may lead to improved trapping of this pest. A food-based pheromone lure was developed for storage moths that has been used with some success for monitoring and possibly control in the US. Cheese mites, Tyrophagus putrescentiae, are serious pests of dried meats, cheeses and other animal-food products. Recent work on semiochemicals for deterring mites from infesting foods, and also work on mite attractants for use in monitoring mites in traps, is underway. Thus semiochemical-based methods have potential to contribute greatly to IPM for stored-product pests, and population suppression without routine use of fumigants and residual insecticides is possible.

Keywords:*pheromones, food odors, traps, mating disruption, Tribolium, Plodia, mold mites*

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Mating disruption for control of stored product insect pests

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During the last decades, mating disruption has been successfully evaluated for the control of numerous field pests, especially Lepidoptera. However, until recently, the data available for the application of mating disruption is stored product pests were relatively few, and mostly restricted to laboratory studies. Nevertheless, the stored-product ecosystem can be regarded as an ideal environment for the application of this technique for two basic reasons. First, it is an enclosed environment, which means that immigration of mated females from outside is rather limited, at least in comparison with field pests. Moreover, most stored product Pyralidae respond successfully to one single pheromone, (9Z,12E)-tetradecadienyl acetate (known as TDA or ZETA), which means that the control of many species with only one component is feasible. This feasibility was tested during the last five years in Europe and the US, with various and often contradictory results. A series of field trials, carried out between 2007 and 2012, indicate that pheromone-based control of stored product Pyralidae is effective, and can be used in various types of facilities and conditions, without loss in efficacy. Currently, several mating disruption formulations are commercially available in many parts of the world, but additional testing to standardize its use is needed in some cases. Apart from the "classical" mating disruption formulations, the use of "auto-confusion" with TDA was also evaluated, and recently commercialized. Overall, the use of mating disruption for the control of stored-product Pyralidae is an effective, viable and non-toxic method over the use of traditional pesticides.

Keywords:*mating disruption, auto confusion, Pyralidae, stored product protection*

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Synthetic pheromone-based mating disruption for cigarette beetles: Present and future perspectives

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Each year, the cigarette beetle, Lasioderma serricorne (Coleptera: Anobiidae) cause significant damage to multi-billion dollar food and feed industries nationwide. These beetles are pervasive and commonly associated with flour mills in the United States. A non-chemical alternative in managing L. serricorne is the application of mating disruption, in which an unnaturally high level of synthetic sex pheromone is released in an area that results in males failing to locate females with an ensuing population crash. Using synthetic anhydroserricornin and serricornin, the sex pheromone of L. serricorne, mating disruption trials were conducted in selected food and feed processing facilities in South Carolina during 2010 and 2011. Mills subjected to mating disruption trials were monitored using oviposition cups and pheromone traps lured with monitoring pheromone. Immediately after deployment of mating disruption dispensers, population levels indicated a "significant trap shut down" effect in treated facilities. A significant reduction was observed in number of adults beetles caught in the traps eight weeks before and after treatment in year 2010 and 2011. Adults emerged from oviposition cup also decreased soon after mating disruption treatment. Preliminary field studies in the U.S. suggest that release of the synthetic sex pheromone of L. serricorne can significantly inhibit proper orientation of male cigarette beetles to females and results in reduced reproduction.

Keywords:*Lasioderma serricorne, Mating disruption, Stored-products, Pheromone*

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Influence of interspecific competition between stored products pests on biological control with parasitoids

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Interspecific competition between pest species sharing common resources would be an important factor in suppression efficiency by their parasitoids because the efficiency could be modified by the competition between the pests or by the interaction with the other parasitoid. In this study, to evaluate the influences of the competition on biological control with parasitoids, five combinations were used to test the influences: 1) single population of rice weevil and Indian meal moth 2) mixed population of rice weevil and Indian meal moth 3) the mixed population under A. calandrae 4) the mixed population under B. hebetor and 5) the mixed population under both parasitoids. In mixed population of rice weevil and Indian meal moth, the interspecific competition between rice weevil and Indian meal moth affected the moth population positively, but negatively the rice weevil population, suggesting that indian meal moth has higher competition ability on both resource use and interference competition than rice weevil. A. calandrae or B. hebetor alone could not change the population dynamics of the two competing pests with similar densities of the rice weevil and Indian meal moth as those without the parasitoids. When both parasitoids occurred together in the mixed populations, the population dynamics of the rice weevil and Indian meal moth were more stable than in the other combinations with significantly lower density level, which could be due to the changes in the spatial coincidence caused by the interspecific interaction between the two parasitoids.

Keywords:*Anisopteromalus calandrae, biological control, Bracon hebetor, Interspecific competition, Plodia interpunctella, Sitophilus oryzae*

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Practicality of using cold treatments for control of stored-product insect pests

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Lowering the storage temperature below an optimum threshold will reduce damage caused by insect pests. Stored-product insects are generally unable to reproduce below 18℃ and cannot move below 5℃, whereas temperatures between 15℃ and -15℃ will eventually kill them. The lower the temperature, the faster the insects will succumb to cold injury. Stored-product insects are unable to survive freezing between -10℃ and -18℃. Species, sex, developmental stage, temperature level, relative humidity, product moisture, acclimation, product type and other factors that determine pest susceptibility to cold are examined. Low temperature strategies as well as existing and potential technologies that might be considered for cold treatments in storage facilities such as ambient aeration, chilled aeration, grain turning, freezing etc. are described. The mode of action, cost, effectiveness, practicality, limitations, hazards, integration with other IPM methods and possible areas for future research for cold treatments in storages are investigated. The main goal of this review is to demonstrate how cold treatments can practically prevent or control insect infestations in stored products focusing on grain and seeds, dried fruits and nuts, flours, and processed and packaged foods.

Keywords:*stored product insects, integrated pest management, cold treatment, extreme temperature*

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Occurrence and level of phosphine resistance in stored-product insects in Oklahoma USA

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Phosphine gas, or hydrogen phosphide (PH3), is the most common insecticide applied to durable stored products worldwide and is routinely used in U.S. for treatment of bulk-stored cereal grains and other durable stored products. Research from the late 1980s revealed low frequencies of resistance to various residual grain protectant insecticides and to phosphine in grain insect species collected in Oklahoma. The present work, which employed the same previously established discriminating dose bioassays for phosphine toxicity as in the earlier study, evaluated adults of nine different populations of red flour beetle, Tribolium castaneum, and five populations of lesser grain borer, Rhyzopertha dominica, collected from broad geographic locations in Oklahoma. One additional population for each species was a laboratory susceptible strain. Discriminating dose assays determined eight out of the nine T. castaneum populations, and all five populations of R. dominica, contained phosphine-resistant individuals, and maximum resistance frequencies for these species were 94% and 98%, respectively. Dose-response bioassays and logit analyses determined that LC99 values were approximately 3 ppm for susceptible and 377 ppm for resistant T. castaneum, and approximately 2 ppm for susceptible and 3,430 ppm for resistant R. dominica. The most resistant T. castaneum population was 119-fold more resistant than the susceptible strain and the most resistant R. dominica population was over 1,500-fold more resistant. Results suggest a substantial increase in phosphine resistance in these major stored-wheat pests in the past 20 yr, and these levels of resistance to phosphine approach those reported for other stored-grain pest species in other countries.

Keywords:*Phosphine resistance*

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Practical implications of insecticide resistance in stored-product insects

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Stored-product protection in warmer climates is largely dependent on insecticide use. The ever increasing safety and environmental concerns added by requirements of high control efficacy imposes restrictions to these compounds and how they can be explored. Furthermore, insecticide resistance is a frequent problem among these insect pest species. This phenomenon is a serious concern in itself since it increases control costs, impairs control efficacy and may lead to insecticide overuse consequently increasing their risk to human and environmental safety. In addition, the expression of insecticide resistance is potentially associated with sub-lethal effects, particularly behavioral effects, which can affect the relationship of the target species and other components of their environment, including natural enemies and other insect pest species. Such components may in turn exhibit indirect effects on the target species under an insecticide-impregnated environment. The sub-lethal effects may even favor higher rates of pest population growth in the target species and the insect behavior when exposed to such compounds may lead to avoidance of toxic exposure. As sub-lethal exposure is likely under practical conditions of insecticide use in stored products and the co-occurrence of different pest species and their natural enemies is expected in such conditions, they should be considering when assessing the impact of insecticide resistance in stored product insects since they are likely to compromise even further insecticide efficacy and their associated problems.

Keywords:*Insecticide resistance, stored product insects, hormesis, sub-lehtal exposure*

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S1202TU16

Urban, Stored Product and Post Harvest Entomology

Recent advances in fumigation for control of insect pests in stored grain

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Phosphine has been scheduled for a toxicological and residue Re-Evaluation Review in 2014 by the FAO Codex Alimentarius organisation that sets internationally agreed maximum residue limits (MRLs) for pesticides. Murdoch University Postharvest Plant Biosecurity Research Laboratory has investigated the fate of 32P labelled phosphine in grain and grain fractions. Radioactively-labelled phosphine is a useful and sensitive tool to study residue of phosphine in grains and their fractions. This research will assist industry to conduct good phosphine fumigation practice. Fumigation of grain, fruit and empty cells with ethyl formate has been evaluated and different formulations, eg., liquid ethyl formate, ethyl formate (95%) + MITC (%%) and ethyl formate (16.3%) + CO2 (83.7%) and different application methods has been developed. Ethanedinitrile (EDN) is a methyl bromide (MB) alternative fumigant has been shown to have potential as a quarantine treatment for timber. Unlike MB, SF and PH3, the penetration of EDN into timber blocks occurred with and across the grain of the timber block. Ethanedinitrile is also a great fumigant for soil to control insects, nematodes, pathogens and weeds. It could be potential to replace MB as a fast fumigant for pre-shipment treatment and for devitalisation of imported grain in Australia. ProFume is Sulfuryl fluoride (originally developed by the Dow Chemical Company) registered for fumigation of grains. As a fumigant, it shows low sorption on grain, non-flammable gas, and with highly toxic to the most stored grain insects at adult stage, but egg stage is more tolerance.

Keywords:*Fumigant, fumigation, stored grain, stored product insect pests*

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S1202TU17

Urban, Stored Product and Post Harvest Entomology

Recent advances in fumigation for control of insect pests in dried fruits and nuts

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United States agricultural industries are facing, with increasing frequency, environmental and pest-related food safety requirements that are fundamentally difficult to balance. Failure to properly sanitize foodstuffs in trade and marketing channels can result in insect- and microbial-derived damage that limits economic profitability, curtails market access, and vectors plant, animal, and human illnesses. Despite a historic precedence of effectiveness, the use of chemicals, including fumigants, for disinfestation and disinfection of foodstuffs is under close regulatory supervision due to consumer consciousness regarding unintended health effects resulting from chemical residues. Regardless of personal viewpoints, or merit of scientific backing, chemical treatment advocacy and opposition is expected to continue. This presentation describes experimental infrastructure and critical research elements of the Crop Protection and Quality Research Unit of the USDA-ARS-SJVASC that specifically address this contemporary dilemma, one that will only become more challenging and important as the role of the United States in feeding the world expands over the next decades. In particular, recent developments in postharvest chamber fumigations are presented, such as methyl bromide and alternative phytosanitary treatments for insect control, strategies to limit or reduce emission of fumigants to the atmosphere, and novel technologies for residue removal.

Keywords:*postharvest fumigation, methyl bromide alternatives*

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Current status of non-chemical methods for control of insect pests in dried fruits and nuts

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Regulatory agencies and consumers have no tolerance for live insects in dried fruit and tree nut commodities; consequently, successful marketing of these products requires postharvest treatments designed to kill any pest insects. Recent regulatory actions have severely limited the use of chemical fumigants by US dried fruit and nut processors to control postharvest insect populations. Non-chemical treatments would provide an alternative to fumigation, and would also be useful to organic processors. One proposed treatment uses radio frequency energy to rapidly heat walnuts to an average temperature of 60°C for 5 minutes. Large-scale tests using an industrial radio frequency heater successfully disinfested walnuts of navel orangeworm while maintaining good product quality. A second alternative exposes product to a low pressure environment of 50 mm Hg in flexible polyvinyl chloride containers. Temperature and moisture content of the product strongly affects treatment efficacy, with complete mortality of test insects achieved at treatment exposures of 48-72 hours under optimal conditions. Another alternative is the use of cold storage (0-5°C) to either disinfest product or prevent reinfestation of clean product, and may be effectively combined with other non-chemical treatments. At temperatures of 0 and 5°C, 95% mortality of test insects was achieved at 10 and 18 days, respectively. Storage at 10°C prevented reproduction, product damage and reinfestation, but 127 day exposures were required to kill 95% of Indianmeal moth larvae. While these non-chemical treatments are effective, they usually require considerable capital investment and modification of existing processing plants, slowing their adoption by the industry.

Keywords:*dried fruit, tree nuts, radio frequency, low pressure, cold treatment*

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The importance of being postharvest

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Postharvest Entomology is often an afterthought when considering insect pest management strategies of fresh agricultural commodities destined for export markets. Unfortunately, the success of pest management programs for these commodities may be negated due to import restrictions based on quarantine concerns. In these situations, the postharvest entomologist must be flexible and innovative in identifying the pest problem, examining the production, harvest, and processing procedures, and developing the most effective approach to achieve quarantine security while maintaining economic viability of the production system. Many times, attainment of quarantine goals will include a direct postharvest treatment, such as methyl bromide fumigation or irradiation. However, the axiom "no treatment is the best treatment" is a consideration that should be on the forefront of any postharvest entomologist's mind. These 'non-treatment' alternatives to attaining quarantine security can include determination of host status, establishment of areas of low pest prevalence and pest free zones, identification of pathways to facilitate systems approaches, and development of ecological niche models to generate biorational pest risk analyses. All of these approaches, from direct commodity treatments to ecological niche modeling, have been utilized in addressing insect pest quarantine requirements for tree fruits from the Pacific Northwest of the United States of America and will be discussed in this paper.

Keywords:*Postharvest*

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Heat as a postharvest disinfestation treatment for floricultural crops

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Heat in the form of hot water was first used as a disinfestation treatment to treat seed potatoes for late blight in 1882. . Heat treatment has been used to control fungal, bacterial and viral diseases, plant-parasitic nematodes and insect pests, but was shelved for about 50 years with the advent of nerve poisons (DDT, diazinon) and chemical fumigants (methyl bromide). Heat treatments not only control pests and diseases, but are a benefit to certain plants by increasing rooting, budding and vase life. Plants sensitive to heat injury can also be conditioned to tolerate heat treatments. Hot water treatment for 45-49 C (113-120 F) for 10 to 15 min disinfests flowers, foliage, potted plants, propagative cuttings, and media of many pests of quarantine significance, including ants, aphids, mealybugs, scale insects, plant-parasitic nematodes, snails and slugs. Hot water dips of tropical propagative cuttings can be used to disinfest cuttings of insects, nematodes and pathogens with the side benefit of increase in rooting. Hot air at 40 C (104 F) conditions plants, foliage and flowers to tolerate hot water and at 44 C (112 F) controls thrips and other insects. Use of steam to pasteurize (149 F for >30 min) or sterilize (185 F for >30 min) plant media will disinfest the media of fungal and bacterial pathogens and plant-parasitic nematodes. Heat is an effective and sustainable postharvest pest and disease disinfestation treatment for export floricultural crops.

Keywords:*postharvest disinfestation treatment, heat treatments, hot water dips, propagative cuttings, tropical flowers, plant-parasitic nematodes, scale insects, mealybugs*

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Oxygenated phosphine fumigation for postharvest pest control

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Oxygenated phosphine fumigations (fumigations under superatmospheric oxygen levels) were found to be significantly more effective than the regular fumigations under the normal 20.9% O2 against different life stages of all six insect species tested including western flower thrips (Frankliniella occidentalis), leafminer (Liriomyza langei), grape mealybug (Pseudococcus maritimus), Indian meal moth (Plodia interpunctella), light brown apple moth (Epiphyas postvittana), and lettuce aphid (Nasonovia ribisnigri) at low temperatures. Oxygen level of 30-80% were tested. Oxygenated fumigations under 60% oxygen resulted in significant reduction in treatment time and phosphine concentration to control the insects as compared with the regular phosphine fumigation. Oxygenated fumigation was also effective against eggs of grape mealybug and light brown apple moth which were tolerant to regular phosphine fumigations. Oxygenated phosphine fumigation was also compared with the regular fumigation with cylinderized pure phosphine to control lettuce aphid on lettuce. The oxygenated phosphine fumigation was significantly less phytotoxic to lettuce than the regular phosphine fumigation due to reduced treatment time and low phosphine concentration. Past research also shows that oxygen has ignition suppression effects on phosphine. Therefore, oxygenated phosphine fumigation can be conducted safely. Oxygenated phosphine fumigation seems to have several advantages over the regular phosphine fumigation and have a great potential to develop more effective and safer treatments for postharvest pest control on both fresh and durable commodities.

Keywords:*Phosphine fumigation, postharvest pest control, phytotoxicity, quarantine*

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S1203TH01

Urban, Stored Product and Post Harvest Entomology

Pest ants of urban importance

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In recent years, the importance of ants as pests in urban environments has gained considerable attention. Their importance in the US is only second to termites. Of the some 11,800 species described, a relatively small number have readily adapted to urban environments and become significant pests. In the US, about 50 species are regularly encountered around structures, most not serious pests. Many of the serious pests are considered to be so-called "tramp ants" such as Argentine ant, red imported fire ant, Pharaoh ant, and little fire ant. These exotic species thrive in urban disturbed habitats and have the ability to displace native species. Other native species such as carpenter ants, odorous house ants, and field ants may invade certain urban settings especially in situations where structures are close proximity to native habitats. Stings and bites of certain species have always been of medical concern, but in recent years, more attention has been given to ants that serve as mechanical potential vectors of human disease or may pose an asthma problem. The widespread use of sprays and granular insecticides around the perimeters of structures to control ants has raised concern about its impact on insecticide runoff into urban waterways.

Keywords:*pest ants, urban environments, invasive species, pesticide runoff*

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Urban, Stored Product and Post Harvest Entomology

Argentine ants - understanding their behavior for effective management

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University of California, U.S.A.

The Argentine ant (Linepithema humile) is one of the most destructive invasive ant species around the world. Conventional spray and bait strategies are among the common practices used to control Argentine ants in urban settings. Because of current concerns about environmental contamination and non-target impact by the insecticides, however, there is a greater need for better techniques which would allow us to accomplish better control with less amount of insecticide applied into the environment. Several behaviors of Argentine ant have important implications for the better use of insecticide, and studies implicate that some of these behaviors potentially can be exploited to develop better pest management techniques. First, the efficacy of insecticide application can be improved by maximizing horizontal transfer of the insecticide. Some insecticide has greater degrees of horizontal transfer among individuals of ants via physical contact such as mutual grooming and necrophoresis (dead-carrying behavior). Second, a novel bait station that is referred to as Virtual Bait Station is developed by exploiting mass recruitment behavior to aqueous sugar bait. In the Virtual Bait Station, the sugar water bait does not contain insecticide, but a non-repellent insecticide deposit is placed around the bait, so that ants expose themselves while foraging and recruiting to the bait. Third, efficacy and target specificity of existing control methods for pest ants might be improved by exploiting the target species' innate trail-following behavior, while simultaneously providing environmentally and economically favorable integrated pest management strategies. Specific application of the ideas and future improvement will be discussed.

Keywords:*Argentine ant, Linepithema humile, insecticide, bait, pest ant, spray, virtual bait station, trail pheromone*

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Urban, Stored Product and Post Harvest Entomology

Urban pest ants of South East Asia and their management

Chow-Yang Lee
Universiti Sains Malaysia, Malaysia

Urban pest ants are becoming an increasing important group of urban pests in South East Asia. Important indoor and outdoor species of pest ants include Monomorium pharaonis, Tapinoma melanocephalum, Monomorium destructor, Monomorium floricola, Anoplolepis gracilipes, Pheidole sp. and Paratrechina longicornis. Until recently, the management of pest ants in South East Asia has always relies on the use of insecticides especially from the pyrethroid class, in the form of residual sprays. Recently, baiting becomes a popular mode of ant control. In this presentation, discussion will be made on the common species of urban pest ants of South East Asia, pest management options and the underlying challenges.

Keywords:*Pest ants, South East Asia, ant management, baiting*

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History of invasive ant management with recommendations for improvement

Benjamin Hoffmann
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Invasive ant management has a poor track record. Here I provide an overview of all published ant eradications globally since the banning of organochlorines and identify what I see as the actions that will provide the greatest immediate enhancement of invasive ant management.

Keywords:*ant, eradication, management, adaptive management, invasive, invasive ant*

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Native invasive ants: A novel evolutionary trend in the age of global change

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Purdue University, U.S.A.

Disturbance resulting from urbanization is a leading cause of biotic homogenization worldwide. Native species are replaced with widespread nonnative species and ants are among the world's most notorious invaders. To date, all documented cases of ant invasions involve exotic introduced species that are spread around the world by human-mediated dispersal. I investigated the effect of urbanization on the evolution of invasive characteristics in a native ant species, the odorous house ant, Tapinoma sessile (Say). Colony social structure, life history traits, and the spatial pattern of nest distribution were compared by sampling T. sessile across a gradient of three distinct habitats: natural, semi-natural, and urban. Results demonstrate a remarkable transition in colony social and spatial structure and life history traits between natural and urban environments. In natural habitats, T. sessile colonies are comprised of small, monogyne (single queen), and monodomous (single nest) colonies. In urban areas, T. sessile often exhibit extreme polygyny and polydomy, form large supercolonies, and become a dominant pest. Results also suggest that urban T. sessile colonies may have a negative impact on native ant abundance and diversity. In the natural environment T. sessile coexisted with a wide array of other ant species, while very few ant species were present in the urban environment invaded by T. sessile. Habitat degradation and urbanization can lead to extreme changes in social and spatial colony structure and life history traits in a native ant species and can promote the evolution of invasive characteristics such as polygyny, polydomy, and supercolonial colony structure.

Keywords:*invasive ants*

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Urban, Stored Product and Post Harvest Entomology

The perspective of red imported fire ant control in Taiwan: past, present, and future

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The red imported fire ant (RIFA), Solenopsis invicta, an exotic invasive species that was inadvertently introduced into Asia-Australian regions from USA in early 21st century, was reported in Taiwan in 2003. The initial infestation by RIFA is estimated up to 50,000 ha with most of colonies are found in one of two main counties (Taoyuan and Chiayi). The distribution of S. invicta in Taiwan reveals its invasive nature that the fire ant invasion is associated with global travel and transportation given that the main international airport is located in Taoyuan and that Chiayi harbors one of largest nursery stock industry in Taiwan. To eradicate this pest ant species, the National Red Imported Fire Ant Control Center (NRIFACC) was then launched in 2004 to conduct the surveillance, monitoring, public education, movement restrictions and chemical treatment. As estimated, approximately 23 million USD has been invested in this program in Taiwan since 2004, and infestation in Chiayi seems to be reduced in size substantially but not Taoyuan. The gradual spread of fire ants in Taoyuan indicates the long-term containment might be an appropriate strategy to manage ants there, considering the size of infested area and financial pressure due to tight budget. Hot spot treatment in core area, along with bait broadcast in outlier area, is therefore the main control scheme in North Taiwan this year. In addition, other control agents (e.g., biological control by nature enemies) are now under evaluation and may incorporate into future control/eradication program.

Keywords:*Solenopsis invicta, red imported fire ant, eradication program*

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Advancements in baiting for management of pest ants: *Camponotus* spp *Liometopum* spp and *Tapinoma sessile*

Laurel Dianne Hansen
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Structurally damaging ants occurring in the northern tier of states in the United States and Canada are carpenter ants, Camponotus spp., and in western United States and Canada, velvety tree ants, Liometopum spp. Tapinoma sessile, is also a major nuisance pest occurring in these areas. These species from two different subfamilies differ in biology and behavior but both feed on honeydew, homopteran insects, and other insects. Because of the similarity in feeding requirements, baiting strategies for these ants have proven effective in laboratory and field trials in recent years with baits formulated as gels, granulars, liquids, and in containerized stations. Consumer perception that baits are more environmentally friendly than other management strategies has encouraged the use of baits by professional technicians. Time required by technicians in application and monitoring baits has been reduced with the development of gels that are effective for several weeks, remain acceptable to foraging ants, and can be placed along foraging trails in concealed locations. In large colonies, liquid baits can be dispensed in larger volumes for continuous feeding. Gel and liquid baits are also acceptable early in the season when ants are overwintering in structures and attracted to environments with moisture.

Keywords:*bait*

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Urban, Stored Product and Post Harvest Entomology

Recent development in bait and trap technologies for yellow jacket control

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Yellow jackets are important urban pests worldwide causing human suffering as well as ecological damages. There is a long history of attempt to develop a bait for their control but such efforts have not produced a marketable product. Trapping is currently the most popular control method but it fails to significantly suppress yellow jacket population level. Recently, we have initiated studies to develop bait and trap technologies for more effective yellow jacket control. We have developed a shelve stable ready-to-use yellow jacket bait that is highly attractive and palatable. Preliminary study found that using the bait containing an active ingredient resulted in a significant population reduction in the field.

Keywords:*Yellowjacket, bait, palatable, control, population suppression*

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Preliminary studies on the development of a liquid bait for bed bugs

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Recently, the bed bug, Cimex lectularius L. (Hemiptera: Cimicidae), has re-emerged as a serious and growing problem, not only in North America, but globally. Today the mainstay for bed bug control is limited to the use of insecticide sprays based mainly on pyrethroids. However, recent laboratory studies reported that some bed bug populations have become highly resistant to this group of insecticides. Failure to eliminate resistant bed bugs could be a contributing factor for the spread of this pest. Therefore, alternative effective methods for bed bug control are of great importance. Here we explained the concept of a liquid bait system against bed bugs and the different steps that are being carrying out for its development. Progress toward this goal includes identification of phagostimulants and evaluations of oral toxicity of non-pyrethroid insecticides to bed bugs. The development and use of these technologies could play an important role in reducing human suffering caused by bed bugs, reducing insecticide applications, and minimizing economic losses associated with infestations.

Keywords:*Bed bug, Cimex lectularius, bait systems, phagostimulants, insecticide, oral toxicity*

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Exploiting host molecules to augment the virulence of mycoinsecticides

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Mosquitoes and fire ants represent two of the world's most important pest problem challenges, whose control remains elusive by current chemical pesticide or other means. Biological control by insecticidal fungi is a promising tool in suppressing insect populations; however their low virulence represents a major obstacle to their effective use. We report two examples of a novel approach to insect control, in which expression of host molecules in an insect pathogen was exploited for selective augmentation of virulence. First, expression of the yellow fever mosquito Aedes aegypti trypsin modulating oostatic factor (TMOF) by the entomopathogenic fungus Beauveria bassiana increased fungal toxicity towards mosquito adult and larvae. Larval development was stunted due to anorexia and adult fecundity was reduced because of inhibition of blood digestion. Increased fungal virulence was Aea-TMOF specific; expression of the grey flesh fly Neobellieria bullata (Neb)-TMOF in B. bassiana did not increase toxicity towards mosquitoes and Aea-TMOF expressing strains did not show increased virulence towards flesh flies. Second, expression of the fire ant pyrokinin β-neuropeptide (β-NP) by B. bassiana increased fungal virulence towards fire ants, but did not affect virulence towards the lepidopteran, Galleria mellonella. Ants killed by the β-NP expressing fungus were disrupted in the removal of dead colony members, i.e. necrophoretic behavior. Furthermore, synthetic C-terminal amidated β-NP but not the non-amidated peptide had a dramatic effect on necrophoretic behavior. These data link chemical sensing of a specific peptide to a complex social behavior. The described strategy can be exploited to increase the potential of biological

Keywords:*mosquito, fire ant, biological control, Beauveria bassiana*

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Disruption of ant trail-following behavior with a high dose of synthetic trail pheromone

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Ants are significant urban pests. Currently, control of pest ants relies mainly on insecticidal baits. However, insecticidal baits cannot provide real solution for some species, due to rapid recovery of population. We have tried to control the invasive Argentine ant Linepithema humile in Japan using synthetic trail pheromone. In ants, trail formation via trail pheromone is an important social behavior that enables efficient foraging and migration. Here I present our five main results and propose two novel approaches for ant management, i.e., foraging suppression and migration inhibition via disruption of trail-following behavior. 1) Trails of Argentine ants were disrupted when a high dose of synthetic trail pheromone was dispersed in the habitat atmosphere. The effect was species-specific. 2) The trail disruption led to reduced recruitment of Argentine ants to food sources. 3) Hoping that the reduced recruitment of the ants to food sources leads to suppression of colony growth rate, a year-long treatment with synthetic trail pheromone dispensers was conducted on 100 m2 plots of house gardens in an urban district of Iwakuni City, western Japan, but no clear control effect was obtained within the plots. 4) However, combined treatment with synthetic trail pheromone dispensers and insecticidal baits effectively suppressed ant populations within the 100 m2 plots. 5) Under the strategy of inhibiting ant migration with synthetic trail pheromone and suppressing ant population with insecticidal bait, Argentine ants were successfully eliminated from most of the 6.7-ha habitat range in Yokohama Port, eastern Japan.

Keywords:*trail pheromone, communication disruption, insecticidal bait, Integrated pest management, biological invasions, invasive ant, social insect*

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Teaching sustainable techniques for German cockroach control in low-income, multi-unit housing

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Low-income multi-unit housing is continuously plagued with large populations of German cockroaches. With so many effective bait products (and negligible cockroach resistance to baits), how do these populations continue to exist? One reason is that low-income facilities are often mandated to hire the lowest bidder for pest management contracts. The break-even cost for pest control companies in the US is ~$1.00 /minute of technician time. In cases where the pest control contract for the apartment community averages $6/unit, the technician must spend < 6 min in a single apartment to make any profit for his company. In highly unsanitary and cluttered environments, the technician struggles to find places where gel bait can be applied without contaminating non-labeled surfaces. Consequently, the technician does not have the time (or space) to put out enough bait to reduce the cockroach population. In highly infested units in Richmond VA, cockroach populations in apartment units were monitored prior to their quarterly pest control service in June 2011. Three weeks after treatment, the same units were monitored again, only to find that the populations had doubled in size. We hypothesize that the populations were so large, and the amount of bait applied was so small, that the cockroaches annual summer growth cycle was completely unaffected by the pest control service. The purpose of this study was to develop an application method where large amounts of gel bait (60-90 g; enough to impact the cockroach population within a unit), could be placed in a very short period

Keywords:*Cockroach, IPM, education*

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Trojan Horses for target specific urban pest management

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Subterranean termites are dependent on protozoa in the hindguts of workers to efficiently digest wood. Using the Formosan subterranean termite as a model we are developing alternative systems for termite control with reduced use of chemicals by employing a technique known as paratransgenesis. Paratransgenesis uses genetically engineered microorganisms as "Trojan Horses" to express foreign genes in host organisms bypassing the host's defenses. We engineered yeast to express lytic peptide coupled to a ligand. Lytic peptides are part of the innate immune system of insects and kill microorganisms but are fairly safe for higher eukaryotes. The ligand specifically binds the lytic peptide to the protozoa in the termite gut, which are important for cellulose digestion. The expressed ligand-lytic peptide kills protozoa and the termites starve. However, the addition of the ligand makes the lytic peptide less toxic for non-target cells, such as bacteria or gut tissue cells. The engineered yeast was freeze-dried, incorporated in cellulose bait and fed to termites in the lab. Yeast was shown to survive and increase in numbers in the termite gut. Yeast was also transferred from termites which fed on the yeast bait to termites which did not feed on the bait. Protozoa in the termite gut died within three weeks and all termites died within five weeks after feeding on the bait. Since traditional biological control methods usually show little success in the field due to the effective defense mechanisms in social insects, paratransgenesis is a promising technology for enhanced microbial control.

Keywords:*symbiosis, genetic engineering, termite control*

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Advances in drywood termite detection, a comparative review

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Advances in drywood termite detection, a comparative review. Vernard R. Lewis University of California, Berkeley Department of Environmental Science, Policy and Management 137 Mulford Hall #3114, Berkeley, CA 94720-3114. Abstract -I will review and present published findings on advances in drywood termite detection. My comparisons will include termite feeding devices, motion detectors, infrared, and X-ray technologies. Recent published efficacy testing on canine detection will also be included. I will also highlight those studies that include efficacy testing of devices when drywood termite infestations are hidden from view and behind wall coverings. Also critical to the detection of drywood termites are what factors constitution a drywood termite colony and the frequency of colony feeding. Currently, the definition of a drywood termite colony is based on location; infested boards that are widely separated are assumed to contain different colonies. There is no biological definition. Regarding frequency of colony feeding, until recently it was unknown. Molecular genetics of workers conducted in collaboration with North Carolina State University and chemical analyses of fecal pellets conducted in collaboration with the USDA Forest Service were able to separate colonies of drywood termites and could provide a measure of successful or failed treatments. Additionally, I will review the recent findings that drywood termites have uneven feeding patterns throughout a day and during the year. Finally, the combination of several detection devices used in tandem with knowing the diurnal and seasonal pattern of drywood termite feeding will aid in the development of future detection schemes and devices.

Keywords:*drywood termites, detection, feeding, motion, pellets*

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German cockroach (Dictyoptera: Blattellidae) IPM: A cost / benefit analysis

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The long-term costs and efficacy of two treatment methods for German cockroach control were compared in the public housing environment. The "traditional" treatment consisted of monthly baseboard and crack and crevice treatment (TBCC) using spray and dust formulation insecticides. The integrated pest management treatment (IPM) involved vacuuming, followed by monthly or quarterly applications of baits and insect growth regulator (IGR) devices. Technician time and the amount of product applied were used to measure cost in both treatments. Trap catch (24h) was used to determine treatment efficacy. The cost of the IPM treatment was found to be significantly greater than the traditional treatment at the initiation of the test. However, to evaluate the long-term costs of the two treatments over the entire year, technician time and product quantities were averaged over all units treated within the 12-mo test period (total 600 units per treatment). The average per unit cost of the IPM treatment was significantly greater ($4.06) than that of the TBCC treatment ($1.50). Although the TBCC was less expensive than IPM, it was also less effective. Trap catch indicated that populations in the TBCC treatment were static for the first 5 mo of the test and then had a threefold increase during the summer. Cockroach populations in the IPM treatment were significantly reduced from an average of 24.7 cockroaches per unit before treatment to an average 3.9 cockroaches per unit by month 4. The cockroach populations in the IPM treatment remained suppressed for the remaining 8 mo of the test.

Keywords:*German cockroach, IPM, Control*

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Effectiveness of baiting and IPM for managing German cockroaches in apartment buildings

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Cockroach gel baits are dominant products for managing German cockroaches in the U.S. since the late 1990's.They provide high level of German cockroach population suppression with much less environmental impact than liquid or dust insecticides. We evaluated the effectiveness of baiting alone method for German cockroach control using baits containing hydramethylnon, fipronil, dinotefuran, and indoxacarb in multi-unit apartment buildings occupied by low income people. These results were compared with IPM programs that incorporating sanitation, monitoring, decluttering and baiting. Results show both baiting alone and IPM can successfully control German cockroach populations. IPM provided more sustainable control. Concerns of bait aversion development will be discussed.

Keywords:*German cockroach, IPM, Control, Bait*

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Insecticide resistance and its underlying mechanisms in the German cockroach

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Heavy reliance and high frequency of insecticide usage have led to the development of insecticide resistance in the German cockroach. Since 1952 where the first case of insecticide resistance in the German cockroach was reported in Corpus Christi, Texas, USA, numerous cases of resistance have since been reported. Resistance to insecticides in the German cockroach is no longer limited to the conventional insecticide groups such as chlorinated hydracarbon, organophosphates, carbamates and pyrethroids. More recently, low to moderate levels of resistance to compounds from newer groups such as neonicotinoid, oxadiazine and phenyl pyrazole have been detected. In this paper, discussion will be made on the recent cases of insecticide resistance status in the German cockroach, and the underlying mechanism of resistance found in these collected populations.

Keywords:*Insecticide resistance, German cockroach, Blattella germanica, cockroach management*

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Urban, Stored Product and Post Harvest Entomology

History and development of cockroach bait technology and future innovations

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Cockroach baits have had a long and successful history in urban pest control and they have become an important component of integrated pest management programs throughout the world. In order to attract cockroach species to feed on baits, they must have an attractive, highly palatable matrix. The key to success is developing cockroach bait matrices that are readily fed upon by cockroaches, even with competing food resources that may be available. Bait aversion is a potential issue with the bait matrice. In addition, the active ingredient must be nonrepellant, have a slow or delayed mode of action, cause mortality at low doses, and allow for secondary mortality through horizontal transfer of the bait. New active ingredients, matrices, inerts, delivery devices, and other innovations are driving cockroach bait technology to a new standard. The future of cockraoch integrated pest management with cockroach baiting appears to be bright, as new technology is being developed to constantly improve cockroach control in the urban environment.

Keywords:*Cockroach baits, bait matrix, cockroach IPM, horizontal transfer*

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Bait aversion in the German cockroach

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Baits have been the preferred and dominant formulation of choice for performing German cockroach control services since the 1980s. German cockroach control failures due to behavioral aversion to baits, rather than resistance to insecticides, were first reported in the early 1990. Behavioral and feeding aversion to glucose was documented as the underline mechanism. Less than a decade later, German cockroach control failures were again reported from the field to the cockroach gel baits that contain no glucose. Rotations between major commercially available gel baits did not reverse control failures. Researchers identified and speculated fructose and other substances as the culprits, responsible for the resurgence of German cockroach infestations. The author reviewed the history of bait aversion, the differences between insecticide resistance and bait aversion, the feasibility of bait rotation strategy, and research finding on underlying mechanisms and innovative solutions. The author also provided insights and recommendations on cockroach bait aversion researches and management, as part of an effective cockroach IPM program.

Keywords:*Blattella germanica, bait avoidance, glucose aversion, control failure*

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Green pest management approaches against cockroaches

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Green pest management includes the use of least toxic and preferably organic insecticides together with application methods that reduce the use of insecticides and limit human exposure. In addition, cultural control measures such as habitat modification and changes in human behavior can be integrated to provide rapid and sustainable cockroach management. For peridomestic species such as the Asian and smokybrown cockroach, landscape mulch preference studies have revealed locations for either targeted insecticide application or modifications of the landscape. Light attraction studies have revealed the utility of yellow 'bug lights' for providing light while not attracting cockroaches. Modifications of human behaviors such as altering landcsape watering schedules and removing pet food immediately after feeding help to reduce cockroach resources. Removal of outdoor debris and objects in the landscape also aid control measures. Domiciliary cockroach species such as the brownbanded and German, also have prefered habitats and resources within apartments and homes. Alteration, modification, and elimination of these significantly aid cockroach management. Use of least toxic insecticides is critical indoors and in close proximity to humans and pets. Insecticidal baits either enclosed in child-resistant exposure stations or gel and paste formulations applied in cracks and crevices provide rapid reductions of populations. Targeted direct contact use of aerosolized essential oil formulations is effective, but there is no toxic residual. Combining 'clean-out' applications of essential oils with longer term protected baits is an effective management system. Least toxic insecticides used in conjuntion with habitat modification and resource elimination provides superior cockroach management.

Keywords:*Blattella germanica, Blattella asahinai, Periplaneta fuliginosa, Supella longipalpa*

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Survey of bed bug infestation in Southeast Asia and research updates

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Bed bug survey carried out in Malaysia, Singapore and Thailand revealed that the tropical bed bug Cimex hemipterus (Fabricius) is the predominant species in Southeast Asia. Cimex lectularius was found in the northern region of Thailand. Bed bug infestations were common in hotels and public accommodations when compared to residential premises. The three most common locations of infestation within an infested premise were the bedding (31.09%), the headboard (30.25%), and cracks and crevices surrounding the baseboard, wall, or floor (23.53%). In this presentation, discussion will be made on the results of the bed bug surveys in this region, as well as the recent findings on C. hemipterus biology and ecology.

Keywords:*Bed bugs, South East Asia, survey, Cimex hemipterus, behavior, ecology*

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Bed bug infestation and management in Shenzhen, Guangdong province, China

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Nationwide Four-Pest Elimination campaigns (bed bugs being one of the targeted pests) were implemented in China 1970 to the late 1980s. These campaigns succeeded in eliminating bed bug infestations in most communities. Although international and domestic travel has increased rapidly in China over the past decade, there have only been sporadic new infestations reported in recent years. Shenzhen (a city near Hong Kong) experienced significantly higher number of bed bug infestations than other cities. We investigated the prevalence of bed bug infestation and management in Shenzhen through on site inspection and telephone interviews from September 2011 to March, 2012. Surveyed communities included Health and Epidemic Prevention Stations, pest control companies, schools, hospitals, factories and residences. Only tropical bed bug, Cimex hemipterus (F.) was found in the surveys. The common bed bug, Cimex lectularius L. was not identified in this survey, though it was reported that the common bed bug was the dominated species in Shenzhen in the literature. This study indicates that bed bug infestation in Shenzhen dramatically decreased following the campaigns from 1970 to the late 1980s, but remained active in factory dormitories and old rental houses where crowded and poor living conditions were present.

Keywords:*Cimex hemipterus, infestation, management*

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Urban, Stored Product and Post Harvest Entomology

Bed bug management practices in the U.S.A.

Ronald Deane Harrison
Orkin, LLC, U.S.A.

Abstract 2012 ICE 12-4 Bed bug management practices in the U.S.A. Around 2000 AD, bed bugs started appearing as a minor pest in the United States. Traditional spraying of baseboards has been replaced by more targeted pest specific methods of treatment. These IPM methods do not seem to work well for bed bugs. Experienced technicians reverted back to base board type applications. These treatments were not successful either. During the last 10 years, many treatment methods have been tested. Bed bugs have shown resistance to dust and liquid formulated pyrethroids. Pyrethroid only products have been replaced by a different class or combination of pesticide chemistries. Heat is another treatment technique which has been incorporated into bed bug service. Fumigation has been implemented on a limited basis. Education and cultural techniques have been implemented with limited success. Pest control companies in the United States that follow strict treatment protocols are successful in controlling bed bugs, but services that are not thorough allow for pesticide resistance and for individual bed bugs to remain. Certain parts of society do not treat for bed bug infestations thus allowing bed bugs to spread.

Keywords:*Bed bug*

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Urban, Stored Product and Post Harvest Entomology

Evaluation of sustainable methods for bed bug control in low-income facilities

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The common bed bug, Cimex lectularius (L.) is establishing itself as the most economically important pest in the United States. Arguably, the low-income, multi-unit housing industry has been the most financially vulnerable to infestations of this pest. Several states have passed legislation identifying apartment owners as being financially responsible for bed bug eradication. However, bed bug management is a very time consuming process. A highly infested apartment complex can take several months to treat, with new bed bug introductions occurring throughout the treatment process. In the US, apartment management companies have paid anywhere from $8,000 to more than $100,000 in a single year for bed bug treatment. To manage the costs of bed bug remediation, proactive bed bug prevention measures need to be adopted. However, bed bug prevention methods for use in multi-unit housing have not been developed or evaluated for efficacy. The objective of this study was to develop, and evaluate the efficacy of a bed bug prevention protocol in the field. Specifically, we intended to determine if the protocol (which included resident training, vacuuming, applications of diatomaceous earth, installation of passive monitoring devices, and the building of an on-site heat chamber) could be successfully implemented by apartment facilities staff after being trained by certified applicators. Next, we wanted to determine if the implementation of the protocol would reduce the number of infestations and subsequent remediation costs. In 2012, a bed bug prevention protocol was implemented in a 120-unit government subsidized facility located in Harrisonburg, VA.

Keywords:*Bed bug, control*

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Urban, Stored Product and Post Harvest Entomology

Chemical ecology of bed bugs: Practical applications

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Understanding the host-finding behavior of bed bugs is utmost important in designing effective monitoring tools and for controlling bed bugs. A large array of chemicals are found to illicit bed bug responses in laboratory assays. Yet, their practical use in bed bug monitoring was rarely investigated. We evaluated the effect of > 12 chemical lures and their combinations on bed bug monitors in the laboratory. Then we investigated the attractiveness of a chemical lure mixture in naturally infested apartments. Many of the suggested chemicals that affect bed bug behavior in laboratory evaluations did not exhibit values in bed bug monitoring. Nevertheless, an inexpensive lure mixture was found being able to significantly increase the bed bug monitor efficacy when used alone or in combination with carbon dioxide (CO2). It is clear that chemical lures are important behavioral modulators in a short range (i.e. < 1 m) whereas CO2 is the most important attractant for initiating bed bug foraging activities. The results also demonstrate the complex nature of the bed bug responses to chemical lures. Their responses are at least affected by the bed bug hungry levels, distance of bed bugs from the lure source, air flow rate, concentration of lure in the air, and presence/absence of a human host. Further investigations on bed bug responses to various lures will help develop more effective bed bug monitors that can be used to increase the monitor efficacy and reduce monitoring costs.

Keywords:*bed bug, lure, monitor*

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Refining non-chemical control tactics against the bed bug, *Cimex lectularius*

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The resurgence of bed bugs in society and initial difficulties encountered with insecticide applications against this pest has resulted in a number of non-chemical controls being developed that either replace or supplement traditional treatments. Use of extreme temperatures against insect infestations is not a novel approach, but development of commercial equipment and improved deployment of existing devices has increased the use of non-chemical controls against this pest. There are several challenges when applying heat or freezing temperatures to control of bed bugs. With high temperature treatments, determining critical temperatures and investigating bed bug behavioral responses have been important considerations. For control using freezing temperatures, understanding how freeze physiology of bed bugs affects required temperature and time of exposure is important for ensuring maximum efficacy in the shortest time possible. Overcoming these challenges make non-chemical treatments more practical by minimizing costs of control through the reduction of energy use and labor required for successful control. Reducing the disruption to people's lives and minimizing the risk of damage caused by these treatments also help to increase adoption of non-chemical control methods. Mainstream use and the experience gained from applying these technologies provide an opportunity to expand non-chemical control methods against other pests in the urban habitat.

Keywords:*Heat, Cold, Integrated Pest Management, Freezer, Heat Treatment*

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Importance of low level detection and the role of monitoring in bed bug management

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Bed bugs are a major pest in many parts of the world. The ability to detect bed bugs in low numbers is essential in managing the costs associated with management efforts and reducing spread. Failure to detect bed bugs at the onset can result in well-established infestations that are costly to control and promote spread. Similarly, failure to detect bed bugs during the terminal end of a treatment effort can result in premature termination of the control program, population rebound and spread. We used a bed bug intercepting device to evaluate population distribution in apartments, to confirm bed bug elimination after treatments, and to evaluate the bed bug population dynamics in lightly infested (< 10 bed bugs in monitors) apartments. This device was more effective in detecting bed bugs compared to visual inspections in previous studies. We found that 50-90% of the bed bugs were consistently captured away from sleeping and resting areas (beds and upholstered furniture). Bed bugs are more mobile than previously believed and the presence of low numbers of bed bugs is easily missed by visual inspections alone. Low level infestations failed to become established in at least 50% of apartments monitored when left untreated. After the infestations were declared eliminated by the pest control technicians, over 50% of them were still found infested. The study demonstrates the importance of proper placement of monitors for detecting bed bugs, long range movement of bed bugs in structures, and intervention strategies for bed bugs when their numbers are low.

Keywords:*Bed Bug, Monitoring, Control*

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Urban, Stored Product and Post Harvest Entomology

Recent progress in bed bug research in the USA

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Prior to the mid-twentieth century bed bugs, Cimex lectularius L., were fairly common in the USA and other countries. However, after the advent of powerful synthetic insecticides such as DDT in the 1940's, their prevalence greatly decreased, particularly in developed countries, and only sporadic infestations were detected, primarily in dwellings with high occupant turnover and questionable sanitation conditions. Over the last ten years however, a general global resurgence of bed bugs has been reported in Asia, Europe, North America, and Australia. Unlike in the past, bed bugs have been reported in a variety of places including single-family dwellings, apartments, hotels, health care facilities, laundries, theaters, college dormitories, and means of transportation. Bed bug infestations are expected to increase in the coming years, presenting many challenges to the pest control industry and the society in general. Basic and applied knowledge about this urban pest is extremely limited compared to other major pest insects. This presentation shows results of research designed to provide not only practical information for bed bug control, but also to begin to resolve the knowledge vacuum that surrounds this pest. What follows is updated information about locomotor activity in bed bugs, feeding behavior, sublethal effects of insecticides, pyrethroid resistance, synergism of pyrethroids, and evaluation of non-pyrethroid insecticides. The implications of these findings on the management of this pest will be discussed.

Keywords:*Bed bugs, Cimex lectularius, insecticide, resistance, feeding behavior, circadian rhythm*

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Urban, Stored Product and Post Harvest Entomology S1206

Beating the bugs: The australian approach to combating the bed bug resurgence

Stephen Lindsay Doggett
Westmead Hospital, Australia

Australia, particularly since the year 2000, has undergone a massive resurgence in bed bug infestations from both the common (Cimex lectularius L.) and tropical (C. hemipterus F.) species. A three point strategy was implemented to combat the rise: the development of an industry standard that promotes 'best practice' pest management, education of stakeholders in 'best practice', and research to evolve 'best practice'. An industry standard that encompasses IPM in bed bug management, 'A Code of Practice for the Control of Bed Bug Infestations in Australia' (CoP), was first developed in 2006 and has been regularly updated with advances in management technologies such that the current edition is the eighth version. This standard has been adopted by other pest management industries and formed the basis of the European bed bug management standard. To encourage education of stakeholders in 'best practice', the CoP is available free from a dedicated web site, www.bedbug.org.au. A curriculum was developed, based on the CoP, and the ensuing course has been used to train stakeholders in bed bug eradication. Pest managers who successfully complete the course are listed on the above bed bug web site. To assist those industries who provide accommodation, a 'Bed Bug Management Policy & Procedural Guide for Accommodation Providers', has been developed and is also freely available. Various research projects have been undertaken, from non-chemical control investigations to insecticide efficacy, with the results used to develop the industry standard. These strategies will continue to be refined while bed bugs remain problematic.

Keywords:*Cimex, Australia, Management*

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Functional characterization of intracellular apoLpo-III in response to *Plasmodium* invasion in mosquito

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Apolipophorin-III (ApoLp-III) is known to play an important role in lipid transport and innate immunity in lepidopteran insects. However, there is no evidence of involvement of ApoLp-IIIs in the immune responses of dipteran insects such as Drosophila and mosquitoes. We report the molecular and functional characterization of An. gambiae apolipophorin-III (AgApoLp-III). Mosquito ApoLp-IIIs have diverged extensively from those of lepidopteran insects; however, the predicted tertiary structure of AgApoLp-III is similar to that of Manduca sexta (tobacco hornworm). We found that AgApoLp-III mRNA expression is strongly induced in the midgut of An. gambiae (G3 strain) mosquitoes in response to Plasmodium berghei infection. Furthermore, immunofluorescence stainings revealed that high levels of AgApoLp-III protein accumulate in the cytoplasm of Plasmodium-invaded cells and AgApoLp-III silencing increases the intensity of P. berghei infection by five fold. There are broad differences in the midgut epithelial responses to Plasmodium invasion between An. gambiae strains. In the G3 strain of An. gambiae AgApoLp-III participates in midgut epithelial defense responses that limit Plasmodium infection.

Keywords:*apolipophorin-III*

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The mosquito STAT pathway and its role in *Plasmodium* development

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Signal transducers and activators of transcription (STAT) family of transcription factors are evolutionary conserved and participate in immunity in response to cytokine signaling. In most of the insects, single STAT gene has been reported; however in Anopheles gambiae gene duplication takes place and ancestral STAT-A gene is regulated by intronless STAT-B. Plasmodium infection induces mRNA expression of SOCS (Suppressor of cytokine signaling) and NOS (nitric oxide synthase). AgSTAT-A silencing reduces the expression of NOS and SOCS suggesting that these genes are regulated by the STAT pathway. The AgSTAT-A pathway mediates a late phase anti-Plasmodial response through the induction of NOS which eventually reduces oocyst survival in An. gambiae. The STAT pathway is conversed in mosquitoes and regulates virus, bacteria and Plasmodium infection. We are exploring further the role of STAT pathway in Indian mosquitoes (An. stephensi) and characterizing the downstream signaling molecules which regulate innate immunity against various pathogens.

Keywords:*Anopheles, STAT, NOS, Plasmodium, bacteria*

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Modulation of mosquito immunity during *Plasmodium* development

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Mosquito gut is not only involved in digestion but also considered to be an immune organ because it encounters foreign antigens present in the ingested food. Gut immunity is under the regulation of some specific molecules which control the effectiveness of immune system against selective foreign antigens. We found that mosquito peroxidases help in creating a barrier between engulfed antigens and gut immunity to suppress their direct encounter. This barrier system modulates the mosquito immunity to maintain low-immunity zone in the gut for the proliferation of commensalisms bacteria and Plasmodium takes an advantage of this environment to complete its development inside the gut lumen. Manipulation of these immunimodulators may be promising to effectively block malaria parasite development.

Keywords:*Gut immunity, peroxidase, Plasmodium*

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A selective barrier in whitefly associated with vector-specific transmission of Tomato yellow leaf curl China virus

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Tomato yellow leaf curl China virus (TYLCCNV) is known to be transmitted by the whiteflies in the Bemisia tabaci species complex in a circulative, persistent manner. Although virus circulation within B. tabaci has been well recognized, the specificity of virus circulation and transmission involved in different cryptic species of the B. tabaci complex remains largely unknown. Here we report that the invasive B. tabaci Mediterranean (MED) species cannot or only very poorly transmits TYLCCNV while the invasive B. tabaci Middle East-Asia Minor 1 (MEAM1) species can effectively transmit it. However, after continuous feeding on virus-infected plants, the relative levels of abundance of viral genomes in whitefly bodies between MED and MEAM1 were similar, suggesting that both species could ingest and retain TYLCCNV efficiently. Viral DNA tracing experiments indicated that TYLCCNV can cross the salivary glands of MEAM1 efficiently but not that of MED. Quantitative PCR indicated that virus abundance in salivary glands was 15 times higher in MEAM1 than in MED. Fluorescence in situ hybridization results revealed that the TYLCCNV transcripts were present in the midgut of these two species while viral DNA was found in the primary salivary gland of MEAM1 but not that of MED. This further demonstrates that the virus transmission route is blocked at the salivary gland cells in MED.

Keywords:*Tomato yellow leaf curl China virus, B. tabaci Mediterranean, salivary gland*

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Increased insulin signaling in the midgut of *Anopheles stephensi* blocks development of the malaria parasite, *Plasmodium falciparum*

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Insulin and the insulin/IGF-1 signaling (IIS) cascade regulates lifespan and immunity in the mosquito Anopheles stephensi. Exogenous insulin from the vertebrate bloodmeal can stimulate IIS in the mosquito midgut, as can feeding the mosquito physiological concentrations of human insulin. Furthermore, these concentrations of insulin can significantly reduce the mosquito's lifespan. To further explore this phenomenon we engineered an active form of the insulin signaling molecule AKT into the mosquito genome under the control of the carboxypeptidase promoter. This resulted in midgut specific expression of the transgene, but surprisingly not bloodmeal specificity. Increased insulin signaling in the midgut led to a significant reduction in mosquito lifespan comparable to mosquitoes fed human insulin. When challenged with Plasmodium falciparum parasites, heterozygous transgenic mosquitoes had a 75-99% reduction in parasite load compared with non-transgenic siblings. More importantly, homozygous Akt mosquitoes were completely resistant to midgut invasion by the malaria parasite. The reduction in infection prevalence and parasite intensity, coupled with a reduction in lifespan results in a two pronged approach towards controlling malaria. Not only would the mosquito transmit far fewer parasites, but those mosquitoes will not survive as long following the extrinsic incubation period, resulting in fewer infectious bites.

Keywords:*Akt, insulin*

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Community engagement practices and genetically-modified mosquitoes

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Novel technologies for public health applications are fostering analyses and inquiry of the roles of communities in research and what they can expect to receive in exchange for their participation. Community engagement activities addressing the results of these analyses are challenging for new scientific approaches, especially those based on controversial strategies and those in which some risks are poorly understood or determined. For many applications, no explicit body of community engagement knowledge exists from which researchers can derive guidance about approaches most likely to be effective in different contexts. Frameworks have been developed recently that provide starting points for broader discussions of community engagement in global health research, particularly as it relates to the development, evaluation, and application of new technologies. Experiences in a number of countries will be presented highlighting unique and common features, and lessons-learned.

Keywords:*genetic engineering*

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Integrating ecological and anthropogenic mechanisms into a framework for advancing forensic entomology research

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Considerable research in forensic entomology has focused on patterns of insect arrival on bodies, however, few have examined what mechanisms drive these patterns. Without an understanding of these mechanisms, it is not possible to make predictions under different scenarios. However, the basic premise of forensic entomology is using the insect evidence to make predictions as to the associated time line. By changing how we think about the system, by incorporating ecological theory and putting our research into a framework of examining mechanisms, we can identify the critical mechanisms driving assembly. Both natural (ecological) and anthropogenic (man-made) mechanisms are important in death investigations and insect evidence, thus it is vital to not only explore these two categories of mechanisms individually, but also how they interact to drive arrival patterns of insect and the resulting insect evidence. Examples of research from my laboratory will illustrate the role of various ecological and anthropogenic mechanisms and their interaction in driving insect arrival patterns. By framing our research in this manner, we more rapidly identify the critical questions that will advance our field.

Keywords:*Calliphoridae, Blow fly, succession*

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Medical & Veterinary Entomology

Estimating postmortem intervals from development of *Thanatophilus* (Silphidae) beetles

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Thanatophilus mutilatus is a carrion-feeding beetle that is endemic to South Africa. Specimens were individually reared through their entire life cycle at one of seven constant temperatures (15℃; 17.5℃; 20℃; 22.5℃; 25℃; 27.5℃ and 30℃), and the time they took to reach eight different development landmarks was recorded. Body length and body width were measured throughout the above-surface development stages. Individuals grew larger at lower temperatures but took longer to develop. Mortality was 90% at 27.5℃ and 100% at 30℃. Using major axis regression, a standard thermal summation model for predicting development was generated and compared with that of Thanatophilus micans. Thanatophilus mutilatus is a forensically important insect which fills the successional period between T. micans and Dermestes haemorrhoidalis, emphasising that even developmental data derived from sister species cannot be used as surrogate data for unstudied focal species in forensic cases.

Keywords:*Forensic Entomology*

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Population genetics in forensic entomology

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Blow flies (Diptera: Calliphoridae) display a variety of habits, however they are best known for their role in the decomposition of carrion. As yet, there are few studies of their population genetic structure, although such data could contribute to such fields as pest management, taxonomy, forensic entomology and veterinary medicine. Since changes in physiological responses due to geographic variation would theoretically have an underlying genetic component, the discovery of genetically distinct subpopulations would suggest that baseline data for forensic applications should be gathered from each subpopulation. Current research into population genetic methods has focused on using a haploid DNA marker (mitochondrial DNA) with some development into the use of microsatellite loci. My research led to the development of thousands of polymorphic DNA loci: amplified fragment length polymorphisms (AFLP). Population genetic data was collected for two common North American blow flies (Lucilia sericata and Phormia regina). Though the populations were panmictic over a wide geographic scale, individual samples of flies were not a random sample of the population, but rather consisted of a large number of related individuals. The reproductive status of females found gravid females also were related (more so than would be expected if dispersal was random), therefore related females are following the same odor plumes throughout a minimum of two carrion visits (one for a protein meal, and one for laying eggs). These data caution the experimental designs set in place for development studies, as samples of blow flies do not represent a random sample of the population.

Keywords:*population genetics, lucilia sericata, phormia regina, AFLP*

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Standards for high quality research on larval morphology of necrophagous flies

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Larvae of flies are usually the most abundant colonizers of dead human bodies recovered in forensic investigations. Immature stages of flies are frequently collected in casework, but usually a sub-sample of eggs or larvae are reared to the adult stage to enable precise species identification. An alternative approach to identification is the use of molecular methods. However, classical methods of species identification of larval material based on an analysis of morphological characters remain an attractive option. Use of good identification keys supported by a detailed description of larval characters may be cheaper and faster than DNA analyses. Contemporary knowledge of the morphology of necrophagous flies is reasonably good in comparison to other groups of Diptera. Good descriptions are accumulating but many are superficial and provide insufficient useful information for taxonomic purposes. Morphological variability of larvae of necrophagous flies is reasonably low. The combination of available features is restricted mainly to small variations in the morphology of the cephaloskeleton, distribution of spines, position and shape of papillae on the anal division, number of lobes of anterior spiracles and position and shape of posterior spiracles. High quality standards in description of larval morphology should encompass: 1) proper killing and preservation techniques, 2) broad sampling according to population diversity, 3) suitable techniques of preparation for microscopic analyses, 4) detailed illustrative documentation of important structures, 5) statistical analysis of quantitative characters. Consistent abiding to the listed rules would result in providing identification keys that should be easily applicable even in poorly equipped laboratories.

Keywords:*larva, Diptera, morphology, identification, forensic entomology*

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Volatile compounds at initial decomposition as attractants for the blow fly, *Chrysomya megacephala* (Fabricius)

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Flies in the Calliphoridae family are primary insect species that attracted to fresh cadavers. Like other necrophagous insects, they primarily use a volatile chemical cue emitted from decomposing organic matter that can orientate them to a corpse. To understanding the interaction between blow flies and initial decomposing chemical volatiles better, comparative attraction of chemical compounds emitted from fresh and 1-day tainted pork scrap were assessed against virgin and mated C. megacephala, a forensically important blow fly species in many parts of the world, including Thailand. Investigations using a wind tunnel bioassay revealed that 1-day tainted pork scrap had a significantly higher potential for luring virgin and mated flies than fresh products. No significant differences in attraction were found between virgin and mated flies to fresh and to 1-day tainted pork scrap. By analyzing with HS-SPME-GC-MS, a total of 13 compounds were identified. In 1-day tainted pork scrap, the predominant compounds were in the sulfur group (dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide and elemental sulfur), followed by organic acids, ammonia, carbon dioxide and indole. On the other hand, the compound obtained from the fresh product was mainly carbon dioxide, in combination with small amounts of ammonia, hydrocarbons, propanedioic acid and elemental sulfur. Based on these results, it is suggested that a larger number of virgin and mated C. megacephala are attracted to 1-day tainted pork scrap than fresh matter. The correlation between chemicals analyzed in this experiment and other compounds documented previously from human corpse and animal carcass is discussed.

Keywords:*Chrysomya megacephala, attractant, initial decomposition*

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Decomposers of carrion: Structural and functional relationships of micro- and macroorgansisms removing vertebrate carcasses from terrestrial ecosystems

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Carrion decomposition has primarily been attributed to invertebrate consumption, with little attention given to microbial communities. Invertebrate succession patterns and corresponding interactions have been documented for over 150 years. However, there has yet to be a study documenting abundance and succession patterns of bacterial communities during decomposition or relating this information to arthropod succession. Total genomic DNA was used to identify bacterial communities via 454-pyrosequencing. Distinct bacterial community successional trajectories were associated with carcass exposed to (ACC) and excluded from (EXC) invertebrates. By the fifth day of decomposition, Firmicutes was dominant (97%) on ACC carcasses while Proteobacteria (82%) was dominant on EXC carcasses. These data are the first to document bacterial community succession on carrion. A secondary objective was to assess microbial community function in response to carrion insect colonization using metabolic profiling. Significant metabolic profile changes occurred during decomposition. Microbial community metabolic function on carrion with insect access continuously decreased over decomposition thus, demonstrating the potential discriminatory power metabolic profiles have during decomposition. Overall, these data add to the knowledge of driving forces governing decomposition of carrion, provide the first information of bacteria colonizing and utilizing carrion, and their potential ecological interactions with necrophagous Diptera. This work provides a foundation of empirical data that may be useful to improve forensic estimations of how long a body has been exposed for colonization by arthropods, or the period of insect activity that is often similar to the minimum postmortem interval. Additionally, these data provide information about decomposition times preceding invertebrate colonization.

Keywords:*Forensic entomology, Microbial community, Carrion*

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Recent studies on factors affecting larval development in forensically important Australian blowflies

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It is well known that the development of carrion-breeding fly larvae is profoundly influenced by a range of variables, both abiotic and biotic. Such factors include the temperature both of the surrounding air and among the larvae themselves, the physicochemical composition of the substrate on which the maggots are feeding, and competition both within and between species. Recent research has focused on better defining the effects of these variables, with the particular aim of refining estimates of minimum time since death. This paper reports on the results of several novel projects devoted to investigating larval development in Australian blowflies: 1) relationship between maggot mass volume and larval growth: our studies have shown that the growth of larvae is positively correlated with the volume of their aggregation (as distinct from larval density); 2) using CT scanning to infer volumes of larval masses: we have revealed that volumes of maggot masses can be inferred from CT images, thus potentially assisting with estimating mass temperatures; 3) interspecific interactions among blowfly larvae: work to date suggests that such interactions may be more sophisticated than mere exploitative resource competition, thus questioning the applicability of developmental data from species monocultures; and 4) effects of methamphetamine on development: this ubiquitous recreational drug and its metabolites significantly influence fly growth, thus potentially affecting estimates of minimum death time in drug cases.

Keywords:*larvae, development, temperature, competition, drugs*

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Three forensic insights from a reveiw of ecological succession on carrion

Martin Villet
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A recent review of ecological succession on carrion provided new three insights. First, the guild structure of the insect community on carrion can be redescribed in a way that is more meaningful to forensic entomology. Second, the traditional description of decomposition in terms of stages is less useful to forensic entomology than a simple distinction between 'wet' and 'dry' phases of the succession process. Third, revised accounts of the guild structure and succession process that characterize the insect community of carrion allow a more mechanistic account of ecological succession in carrion that can enhance forensic entomology worldwide.

Keywords:*Forensic Entomology, Ecological Succession, Community Ecology*

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Medical & Veterinary Entomology

Welcoming Remarks & Future prospect of northern expansion of *Aedes albopictus* in Japan

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Asian tiger mosquito, Aedes albopictus, is one of vectors of the dengue and Chikungunya viruses and is a common mosquito in Southeast Asian and Far Eastern countries including Japan. During past three decades the distribution areas of Ae. albopictus clearly expand to worldwide by world trading of used tiers. In Japan Aedes albopictus distributed widely in Honshu Island with its northern limits between 38° to 40° degrees north. The factors affecting distribution of the species in Japan were studied using the GIS and showed an annual mean temperature higher than 11 Co is most related to the northern limit. During 1998?2010, larval surveillance was carried out in more than 30 cities and towns including rural areas in Tohoku district, the northern limit clearly moved to northward. The future expansion of the distribution of Ae. albopictus was analyzed using 1-km mesh climate data prospected by MIROC (K1) model in 2035 and 2100. From these analyses, the distribution of the mosquitoes clearly expands in the lowland areas of most northern Honshu district in 2035. Ae. albopictus will also invade to Hokkaido Island crossing the Tsugaru Strait until 2100. Our theory was reconfirmed by the technical report published by ECDC in 2009 entitled "Development of Aedes albopictus risk map" .

Keywords:*Aedes albopictus*

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Aerial vector control following natural disasters in the Southern United States

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Some studies have linked an increasing number of hurricanes to climate change. In the Southern United States, a series of Category 4 & 5 hurricanes since 2005 have caused in their wake significant property damage, loss of life, and generated high numbers of mosquitoes. National and state level public health responses typically involve various vector control measures including the application of insecticides by aircraft over large areas. This paper discusses the type, scope, and results of aerial sprays to control mosquitoes and filth flies following Hurricanes with an emphasis on the U.S. Air Force's methods.

Keywords:*mosquito, pesticide*

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Geographical distribution of larval trombiculid mites in Korea (2005~2007)

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A surveillance of larval trombiculid mites was conducted to clarify the geographical distribution of scrub typhus vectors in Korea by collecting wild small mammals twice a year (spring and autumn seasons) at 24 localities from 2005 to 2007. Among 783 trapped rodents, Apodemus agrarius accounted for 87.4% and the trapping rate at riverside was recorded the highest. A total of 67,325 mites representing 4 genera and 14 species were collected and their chigger index (C. I., No. of chigger mites per one rodent) was 86.0. The predominant species were Leptotrombidium pallidum (52.6%), L. scutellare (27.1%), L. palpale (8.2%), L. orientale (5.6%), and Neotrombicula tamiyai (1.7%). However, the portions of L. scutellare in southern areas including endemic provinces such as Jeollabuk-Do (34.3%), Jeollanam-Do (49.0%), and Gyeongsangnam-Do (88%) were relatively higher than middle areas where L. pallidum was predominant in. Additionally, in autumn season, the density of L. scutellare was increased up to 42.0% while that of L. pallidum was decrease to 37.6%. Now we are performing second periodic surveillance (2011~2013) and trying to develop an alternative method using molecular markers for identification of chigger mites instead of morphological classification.

Keywords:*trombiculid mite, geographical distribution, scrub typhus, chigger index*

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Prefabricated trap: Application to monitor fly populations in global change

Kom Sukontason[1], Ratchadawan Ngoen-klan[2], Kittikhun Moophayak[3], Tunwadee Klong-klaew[4], Kabkaew L. Sukontason[5]
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Prefabricated trap: Application to monitor fly populations in global change Kom Sukontason*, Ratchadawan Ngoen-klan, Kittikhun Moophayak, Tunwadee Klong-klaew, Kabkaew L. Sukontason Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand Inevitably, the effect of global warming will trigger the physiological and/or behavioral responses of terrestrial organisms, thereby increasing their populations, including those of some insects in particular areas. The increment of insect populations, especially in those of medical importance, may affect humans by means of involvement with more vectors of disease pathogens. To understand population changes in medically important flies, population monitoring in the natural environment should be performed regularly by using a sweeping or trapping technique. Although several fly trap models have been constructed and utilized, some of them are impractical for fly survey strategy, for example, traps that are too large and heavy for easily transferring collected specimens to the laboratory for investigation. Therefore, we hereby introduce a lightweight and inexpensive apparatus for assembling a fly trap, consisting of 3 parts: 1) frame, made from polyvinylchloride pipe (1.25 cm in diameter), 2) net for fly collection, and 3) entrance dome. Tests of this novel trap, using fresh and tainted pork viscera as bait in field experiments, clearly indicated the great practicality of this device, both for assembling to form a trap and transporting to and from the laboratory. The flies collected revealed 10 species in the family Calliphoridae, 6 species in the family Muscidae, and 3 unidentified species in the family Sarcophagidae.

Keywords:*fly, trap, population*

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Seasonal and annual occurrence of blow flies (Diptera: Calliphoridae) associated with longan orchards and a paddy field in Southern Chiang Mai, Thailand

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This blow fly survey was conducted during 2009 and 2010 using traps baited with 1-day tainted viscera in two longan orchards and one paddy field in Hang Dong district, southern Chiang Mai, northern Thailand. Seven species from Calliphorinae and Chrysomyinae subfamilies were identified. In two longan orchards, Chrysomya megacephala was the most common species collected (68.86%), followed by Achoetandrus rufifacies (24.08%), Hemipyrellia ligurriens (6.00%), Hemipyrellia pulchra (0.63%), Lucilia cuprina (0.24%), Ceylonomyia nigripes (0.10%), and Lucilia papuensis (0.10%). In contrast, three species; C. megacephala (89.42%), A. rufifacies (5.74%) and H. ligurriens (4.84%), were collected in a paddy field. Considering the three most common species collected - C. megacephala, A. rufifacies and H. ligurriens - the overall adult population of C. megacephala captured in the two longan orchards and paddy field fluctuated throughout the year with no significant differences between seasons. As for A. rufifacies, collection at a longan orchard (site HD2) amounted to significantly low population during the cool season. Temperature was the only factor displaying weak positive correlation with seasonal occurrence of this species. In the paddy field, there was no seasonal pattern for A. rufifacies collected or climatic factors correlating to the number of flies captured. Regarding H. ligurriens, collection in a longan orchard (site HD1) showed a significantly higher population in the rainy season. High relative humidity and low light intensity are the main factors for increasing the number of H. ligurriens captured as the lowest population in the paddy field was observed clearly during the dry season.

Keywords:*Blow Flies, Orchard, Population*

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S1303TU06

Medical & Veterinary Entomology

Outbreak of medically important pest insects in Tsunami affected areas in Tohoku District, Japan

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The Great East Japan Earthquake brought unprecedented damages caused by great Tsunami which was unexpected by even experts of disaster. In the field of medical entomology, uncountable number of flies emerged from putrid shes, brown rice and organic fertilizer which were spread from destroyed storehouses on the shoreline. A number of mosquito larvae were observed at newly appeared standing water bodies, a lot of destroyed septic chambers and tanks related to sewage disposal system at tsunami-stricken areas. The members of Department of Medical Entomology, National Institute of Infectious Diseases and Japan Pest Control Association visited Miyagi and Iwate Prefectures of Tohoku Tsunami-stricken regions in 2011 to survey the medically important pest insects. In this symposium, we report, with pictures and movies, the situation of these nuisance and vector insects which explosively emerged at Tohoku eastern seashores.

Keywords:*tsunami, disaster, earthquake, Tohhoku district, mosquito, house fly*

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Medical & Veterinary Entomology

Life traits of *Aedes mosquitoes* as factors of arboviruses emergence

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Life traits of Aedes mosquitoes as factors of arboviruses emergence Among the >3500 mosquito species described in the world, only a few hundred are able to transmit arbovirus to human. For example, as less than 10 among 920 Aedes species effectively transmit dengue and Chikungunya viruses naturally. Based on recent field and experimental results particularly on Aedes albopictus, we will show the role and relative contribution of the different parameters of vector competence and vector capacity in shaping Aedes-arbovirus interactions and the resulting pathogen transmission dynamics and risk of emergence. These include mosquito life history traits and biology parameters such as population dynamics, longevity, trophic behavior, habitat preference, diapause, genetics, immunity and biotic interactions. The mechanisms and processes underlying the patterns observed in the field should be studied all the way, from molecules to individuals and populations, towards a better understanding of what makes a mosquito vector and how best to control it.

Keywords:*Aedes arboviruses biology*

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The mosquito *Aedes albopictus* and its vectorial status

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The mosquito Aedes albopictus (Skuse) (Diptera: Culicidae), native to Southeast Asia, has extended its geographical distribution, invading new temperate and tropical regions. Its geographic expansion facilitated by increasing trading and touristic activities led to emergence of arboviruses in unexpected regions such as Southern Europe. The species was recorded for the first time in Albania in 1979, then in Italy where it has become the main pest in urban settings. It is now present in all European countries around the Mediterranean Sea. Ae. albopictus was incriminated as a main vector in the first European outbreak of chikungunya in the province of Ravenna (Italy) in 2007 and responsible of autochthonous cases of chikungunya and dengue in the south of France in 2010. Using experimental infections, we found that populations from South of France were as efficient as Aedes aegypti, the main vector, to transmit both dengue and chikungunya viruses. The large numbers of travellers returning from dengue- and chikungunya-endemic regions may introduce frequently the virus which has been proved to be efficiently transmitted by autochthonous mosquito species. Outbreaks due to both arboviruses should be expected in developed countries where Ae. albopictus is established.

Keywords:*Aedes albopictus, dengue, chikungunya, transmission*

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The mosquito immune response modulates vector competence for arthropod-borne viruses

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RNA interference (RNAi) mediates normal growth and development and limits viral infection and transposon activity in eukaryotic cells. RNAi involves 21 to 30 nucleotide RNAs classified as microRNAs (miRNAs), exogenous and endogenous small interfering RNAs (exo- and endo-siRNAs) and Piwi-interacting RNAs (piRNAs). We have shown that the exo-siRNA pathway is the major mosquito innate immune response and is an important modulator of dengue virus type 2 (DENV2) infection of Aedes aegypti, the major DENV vector. Virus-specific siRNAs (viRNAs) produced by Dicer-2 (Dcr2) cleavage of long dsRNA forms of DENV2 RNA are required to initiate the antiviral exo-siRNA response. The size and polarity of viRNAs from DENV-infected A. aegypti mosquitoes and cultured cells indicate that they are products of Dcr2 cleavage of long dsRNA, whereas cultured A. albopictus C6/36 cells generate DENV2-specific small RNAs with characteristics suggesting they originate from the piRNA pathway. We speculate that a single-nucleotide deletion in C6/36 cell dcr-2 and defective expression or function of Dcr2 might explain the robust growth of arboviruses in the C6/36 cell line compared to growth in Aag2 cells. While this result emphasizes the importance of the exo-siRNA pathway in the mosquito immune response, it also suggests that other small RNA pathways serve back-up or ancillary roles in mosquito antiviral defense. We showed that genetic diversity in A. aegypti miRNA as well as siRNA pathway genes correlates with the rate of DENV dissemination from infected midguts (the midgut escape barrier), suggesting involvement of all the RNAi pathways in mosquito vector competence.

Keywords:*Aedes aegypti, dengue virus, RNA interference*

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Medical & Veterinary Entomology

Wolbachia density-dependent inhibition to dengue Virus in three *Aedes* mosquito species

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Wolbachia is a maternal transmitted endosymbiotic bacterium that is estimated to infect up to 65% of insect species. The ability of Wolbachia to both induce viral interference and spread into mosquito vector population makes it possible to develop Wolbachia as a biological control agent for dengue control. While Wolbachia induces resistance to dengue virus in the transinfectedAedesaegypti mosquitoes, a similar effect was not observed in Aedesalbopictus and Ae. polynesiensis, which naturally carryWolbachia infection but still service as dengue vectors. In order to understand the mechanism of this lack of Wolbachia-mediated viral interference, we used both Ae. albopictus cell line (Aa23) and mosquitoes to characterize the impact of Wolbachia on dengue infection. We show that there is a strong negative linear correlation betweenthe genome copy of Wolbachia and dengue virus with a dengue infection completely removed when Wolbacha density reaches a certain level. We then compared Wolbachia density between transinfectedAe. aegyptiand naturally infected Ae. albopictus, and between the wild typeAe. polynesiensis and its transinfected line. All the results support a high Wolbachia density in mosquito somatic tissues will lead to a low mosquito vector competence for dengue virus. We discuss our results in relation todevelopment of novel methods to block disease transmission by mosquitoes carrying native Wolbachia infections.

Keywords:*Wolbachia, dengue, Aedesalbopictus, Aedespolynesiensis, Aedesaegypti*

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Progress in understanding RNA interference (RNAi) as a key control mechanism of arbovirus replication and spread in mosquitoes.

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Replication and spread of arboviruses is not passively tolerated in mosquitoes, but controlled by host responses. A key mosquito innate immune response limiting arbovirus replication is RNAi. During insect antiviral RNAi responses, virus-induced double-stranded RNA (dsRNA; mainly derived from replication intermediates) is cleaved by the nuclease Dcr-2 into double-stranded siRNAs, also called virus-induced small interfering RNAs or viRNAs in the context of viral infection. These viRNAs are integrated into the RNA-induced silencing complex (RISC), which contains Ago-2 as a key effector protein. Ago-2 will retain one strand of the viRNA to bind complementary viral RNA, which is then cleaved. These processes are still poorly understood in mosquitoes and mosquito cells. I will discuss progress that has been made in understanding induction and effector mechanisms that underlie antiviral RNAi responses mainly against a prototype mosquito-borne alphavirus, Semliki Forest virus (SFV). The genetic flexibility of SFV and SFV-derived viral replicons have proven to be particularly useful to further understand antiviral RNAi. These studies have been carried out in mosquito cell lines which retain functional RNAi responses, which allows tight control of infections. The respective roles of key proteins involved in RNAi and viRNA functions will be highlighted.

Keywords:*Arbovirus-mosquito-host response-RNA interference*

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Introduction to symbiotic microorganisms associated with medical, hygienic and veterinary pest arthropods

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Many of notorious pest insects and other arthropods of medical, hygienic and veterinary importance are associated with a variety of symbiotic microorganisms of either obligate or facultative nature. Bacteriocyte-associated, nutrition-provisioning, and obligate bacterial endosymbionts, such as Wigglesworthia and Riesia are known from tsetse flies, lice, bedbugs, etc. Facultative bacterial endosymbionts such as Wolbachia, Rickettsia, Arsenophonus, Sodalis, etc. are found from almost all pest arthropods including mosquitoes, fleas, lice, tsetse flies, ticks, etc. Most of vector-borne human and animal pathogens are regarded as facultative endosymbionts of the vector arthropods. Some facultative endosymbionts like Wolbachia are investigated as promising controlling agents to suppress vector competence of the pest arthropods. Here I present an overview of these relevant issues to prime the symposium. [Supported by BRAIN]

Keywords:*Insects, ticks, symbionts, vector, pathogen, pest control*

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Symbiosis in ticks, and the microbial community associated with South-American tick vector of rickettsiosis

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Ticks are ectoparasites, living on the blood of vertebrates. These arthropods are major vectors of a number of diseases, including rickettsiosis, Lyme disease, tularemia, babesiosis. Symbiosis with microorganisms is important in arthropod vectors: symbionts are known to influence the fitness of the arthropods, their capacity to transmit diseases and their resistance to parasites. Symbiotic microorganisms could be exploited to impair the transmission of vector-borne pathogens (symbiotic control). Only a few studies have so far been published on the symbiotic microbiota associated with ticks. Examples of tick-associated symbionts are the intra-mitochondrial bacterium Midichloria mitochondrii and the pathogen-interfering symbiont Rickettsia peacockii. Amblyomma aureolatum and Amlyomma cajennense are South-American ticks, that vector the spotted fever agent Rickettsia rickettsii. They share the same ecological niche. But, while A. aureolatum shows high susceptibility to R. rickettsii, with high infection rates, A. cajennense displays low susceptibility, with lower prevalence. In order to understand the mechanisms behind the difference in susceptibility between the two species, we investigated their associated microbiota and its reaction to the infection with R. rickettsii. DGGE and 16 rRNA library analysis were carried out on lab colonies of A. aureolatum and A. cajennense, before and after infection with the pathogen. The microbiota of the two species displayed overlapping patterns, but the change in the symbiont structure after the infection was more pronounced in the case of A. aureolatum. For both species, the dominant bacterium was a Francisella-like bacterium, that belongs to an emerging clade of tick-associated symbionts.

Keywords:*Amblyomma aureolatum, Amlyomma cajennense, microbiota, DGGE, 16 rRNA library*

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Midichloria, the bacterium that invades tick mitochondria

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Midichloria mitochondrii is the only intra-mitochondrial bacterium thus far described. This microorganism resides in the cytoplasm and mitochondria of the hard tick Ixodes ricinus, infecting the oocytes and other cells of the female reproductive tissues. In addition to I. ricinus, other ticks have been shown to harbor close relatives of Midichloria. The presence of Midichloria in the reproductive apparatus of female ticks ensures its transmission to the progeny. The location in the mitochondrion is peculiar, in that Midichloria occupies the intermembrane space. This location might suggest different kinds of interactions with the host cell. Relatives of Midichloria are assumed to act as energy-scavenging parasites; some of these have also been shown to possess the complete gene set for the synthesis of heme. We have recently published the genome of M. mitochondrii, whose annotation confirms the presence of the genetic apparatuses for the synthesis of both ATP/ADP translocase and heme. Ticks are assumed to be unable to synthesize this prosthetic group. A function for Midichloria might thus be hypothesized, i.e. the provisioning of heme to the host (as in the case of the interaction between Wolbachia and filarial nematodes). Genome annotation also revealed the presence of genes coding for a complete flagellar apparatus and for the cbb3 cytochrome oxidase (previously unreported in Rickettsiales). Phylogenomics showed that these genes have been vertically inherited from the common ancestor of Rickettsiales and mitochondria. These findings suggest a novel scenario on the origin of the eukaryotic cell.

Keywords:*ticks, symbiosis, Midichloria*

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Lariskella arthropodarum, a novel facultative endosymbiont clade in the Rickettsiales associated with stinkbugs, fleas and ticks

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The alphaproteobacterial order Rickettsiales contains well-known bacterial groups such as Wolbachia, Rickettsia, Ehrlichia and Anaplasma, whose members are associated with a diverse set of host/vector species with various relationships ranging from parasitic/pathogenic through commensalistic to mutualistic. Stinkbugs of the genus Nysius possess a pair of red-pigmented bacteriomes in the abdomen, wherein the obligate endosymbiont Schneideria nysicola is harbored (Matsuura et al. 2012a). In addition, we also identified an array of facultative endosymbionts, Wolbachia, Rickettsia and a novel alphaproteobacterium, designated as Lariskella arthropodarum. Of 343 insects representing 24 populations and 4 Nysius species, 285 insects (83.1%) were associated with Lariskella. Further survey of diverse stinkbugs representing 24 families, 191 species, and 582 individuals detected Lariskella from additional 12 species representing 6 families. Molecular phylogenetic analysis showed that Lariskella-allied endosymbionts are found not only from stinkbugs but also from fleas and ticks, forming a coherent monophyletic group in the Rickettsiales. In situ hybridization and electron microscopy identified small filamentous Lariskella cells in various tissues of Nysius plebeius including the bacteriome and ovary (Matsuura et al. 2012b). The Rickettsiales is known for a number of animal and human pathogens including Rickettsia prowazekii for epidemic typhus, R. rickettsii for Rocky Mountain spotted fever, Ehrlichia chaffeensis for monocytic ehrlichiosis, etc. Notably, Mediannikov et al. (2004) detected a 16S rRNA gene sequence of Lariskella from blood and biopsy samples of acute fever patients bitten by Ixodes ticks. Hence, potential medical relevance of Lariskella should be a focal issue in future studies. [Supported by BRAIN]

Keywords:*alphaproteobacteria, Rickettsiales, Nysius spp., sitnkbugs, facultative endosymbiont*

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Cardinium of *Ixodes scapularis*: Its Characteristics and Genome Sequence

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Cardinium, a facultative symbiont of arthropods and nematodes, has attracted attention as a manipulator of host sex and reproduction since 2001 (Weeks et al.; Zchori-Fein et al.). Cardinium is a member of the Bacteroidetes group and is in a different lineage from Wolbachia, which belongs to alphaproteobacteria and also cause reproductive abnormality in host arthropods. Cardinium of Ixodes scapularis (IsCar) was cultured by chance in a tick cell line in 1996 and identified as being structurally similar to other symbiotic microorganisms found in arthropods. The phenotypes that IsCar may induce in host ticks are not known. We have maintained the IsCar in the silkworm cell line aff3 and examined gene expression of IsCar?infected host cells using a silkworm microarray. A Wolbachia (wStr) from the small brown planthopper Laodelphax straitellus was also cultivated in aff3 cells and we compared gene expression in cells infected with IsCar or wStr. Wolbachia did not up-regulate host insect immune genes whereas Cardinium clearly up-regulated them. Whole genome sequences of arthropod symbionts have been accumulated during these 10 years. Determination of genome sequence of IsCar contributes to the study of host-symbiont interaction, especially manipulation mechanisms of endosymbionts. We prepared genomic DNA from isolated IsCar from the silkworm cell line. Sequences were determined by Sanger sequencing using 2 Kb and 5 Kb shotgun libraries and by pyrosequencing and short-read sequencing. Genome sequence assembly was hindered by the presence of many transposon sequences in the IsCar genome, resulting in 121 sequence scaffolds. [Supported by BRAIN]

Keywords:*Cardinium, Wolbachia, symbiont, Ixodes, genome, microarray*

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Mosquito symbionts as a tool for mosquito borne disease control

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Mosquitoes have a relevant ability to adapt to very different types of habitats, thus they are present all around the world. It is highly likely that the microbiota of the mosquitoes had a key role in establishing their great capacity to adapt to different environments. A deep knowledge of the mosquito?microbiota relationships may give the chance to better understand some traits of mosquito biology and may help the development of innovative mosquito-borne disease-control strategies aimed to reduce mosquito vectorial capacity, with particular regards to those inhibiting pathogen development within mosquito transmission. In this context, since some years my research group at the University of Camerino has started a study for the identification of mosquito symbionts. On the basis of their particular features we have mostly focused our interest on two of them: 1) The acetic acid bacterium Asaia 2) The yeast Wickeramomyces anomalus -Asaia is a Gram-negative α-proteobacterium, found in some mosquito species including several malaria vectors and dengue virus vectors. Asaia is the most prevalent bacteria in both natural and lab mosquito populations and its prevalence often reaches 100%. Asaia has been detected in in the midgut, salivary glands, and reproductive organs of the mosquito. For all these reasons Asaia could has been proposed to express anti-pathogen(s) molecules directly in the midgut of the mosquito to exert an inhibitory effect against pathogens.

Keywords:*Mosquito, Asaia, Yeast, Vector Borne Diseases*

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Medical & Veterinary Entomology

The mosquito gut microbiota in the war against human pathogens

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The resident microbiota of insect vectors can impede transmission of human pathogens. Recent studies have highlighted the capacity of endogenous bacteria to decrease viral and parasitic infections in mosquito and tsetse fly vectors by activating their immune responses or directly inhibiting pathogen development. These microbes may prove effective agents for manipulating the vector competence for malaria and other important human pathogens, as well as representing promising sources of anti-pathogen therapeutic natural products. We have identified a variety of microbes of the mosquito midgut microflora with potent in vivo and in vitro activities against the dengue virus and different stages of the malaria parasite Plasmodium. The potential of these microbes for disease control will be discussed.

Keywords:*Malaria, dengue, mosquitoes, microbiota*

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Wolbachia genes acquired through lateral gene transfer contribute to feeding habit of the host bedbug

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Diverse insects utilize their specific diets. If not all, many of such food adaptations are attributable to their nutrition-provisioning bacterial symbionts. These symbionts are often harbored in specialized cells called bacteriocytes that constitute a symbiotic organ bacteriome. Facultative and parasitic bacterial symbionts like Wolbachia have been regarded as evolutionarily distinct from such obligate nutritional mutualists like Buchnera in aphids. However, we recently discovered that, in the bedbug Cimex lectularius, a Wolbachia endosymbiont resides in a pair of bacteriomes as an obligate nutritional mutualist. The symbiont is vertically transmitted via ovarial passage, elimination of the symbiont resulted in retarded growth and sterility of the host, and these deficiencies were rescued by oral supplementation of B vitamins that are lacking in blood meal of the host. Here we report the complete genome sequence of the mutualistic Wolbachia strain wCle associated with C. lectularius. Gene repertoire and evolutionary patterns in the wCle genome were similar to those of other Wolbachia genomes. Intriguingly, the wCle genome encoded a set of genes for biosynthesis of B vitamins, which are lacking in other Wolbachia genomes. Molecular phylogenetic analysis showed that the vitamin synthesis genes are closely related to those of phylogenetically unrelated bacteria, indicating lateral transfer origin of those genes. In addition, elimination of the specific B vitamin from the artificial diet resulted in retarded growth of aposymbiotic insects. These results strongly suggest that horizontal gene acquisition by the Wolbachia strain might contribute to the blood feeding habit of the host bedbug. [Supported by BRAIN]

Keywords:*pest arthropod, bedbug, endosymbiosis, Wolbachia, lateral gene transfer*

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Characterisation of imidacloprid resistance in the housefly *Musca domestica* (Diptera: Muscidae)

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A housefly population collected from filed (IFS) was selected continuously with imidacloprid to establish the resistant strain (IRS) and near isogenic lines of resistance (N-IRS) and susceptibility (N-ISS) to imidacloprid were built up. The N-IRS possessed about 200 times of imidacloprid resistance compared to the homologous susceptible strain. The level of cross resistance to beta-cypermethrin dramatically decreased from 1175-fold in the IRS to 18-fold in the N-IRS. The N-IRS lost the cross resistance to azamethiphos though the original cross resistance to this insecticide only is 4 times. However, no difference of cross resistance to acetamiprid was observed between the IRS and N-IRS because of the same action mechanism with imidacloprid. Three synergists (piperonyl butoxide, S,S,S-tributylphosphorotrithioate and diethyl maleate) all showed the significant synergism to imidacloprid in N-IRS, indicating that the multiple factors involved in N-IRS resistance to imidacloprid. De novo analysis of transcriptome on both N-ISS and N-IRS of Musca domestica was carried out. The expression levels of 99 of cytochrome P450 genes exhibited upregulation, 111 of cytochrome P450 genes down regulation in the N-IRS. 8 genes showed upregulation and 12 genes down redulation for glutathione S-transferase. 11 genes showed upregulation and 11 genes down redulation for carboxylesterase. 2 genes showed upregulation and 1 genes down redulation for acetylcholinesterase. 17 genes showed upregulation and 31 genes down redulation for nicotinic acetylcholine receptor in the N-IRS. These results are very useful for clarifying the imidacloprid resistance in Musca domestica.

Keywords:*Musca domestica*

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Sustaining malaria control by tackling pyrethroid resistance in *Anopheles mosquitoes*.

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The World Health Organization estimates that there were 216 million malaria cases resulting in 655,000 deaths in 2010. With increasing reports of drug resistance and no vaccine, controlling the mosquito vector is the best method of malaria prevention. This typically involves the use of pyrethroid insecticides, either via insecticide treated bednets or indoor residual spraying. However, the efficiency of these tools is been threatened by the emergence of resistance to insecticides in malaria vectors. Understanding the underlying mechanisms is fundamental for the control of resistant mosquito populations. Resistance to pyrethroids has been frequently associated with a series of mutations in the target-site sodium channel, known as kdr mutations (target-site resistance) and also to an increase metabolism of insecticide by detoxification enzymes (metabolic resistance). Two main mutations occurring at codon 1014 and resulting in the substitution of leucine to serine (kdr East) or phenylalanine (kdr West) are now routinely screened to detect any signs of insecticide resistance in Anopheles populations. Recently, our group discovered a new kdr mutation N1575Y that gives, in association with the L1014F mutation an increase resistance of mosquitoes to DDT and permethrin. In parallel, recent microarray studies on pyrethroid resistant Anopheles strains detected several potential candidate genes, mainly detoxification genes potentially involved in pyrethroid resistance. One of them, CYP6M2, coding for a cytochome P450 monooxygenase has beeen shown to metabolize pyrethroids and DDT. The role of the different kdr mutations and the metabolic resistance on the control of pyrethroid resistant Anopheles will be discussed.

Keywords:*Anopheles, Pyrethroid resistance, kdr mutations, metabolic resistance, transcriptomics*

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Spread of overexpressing CYP9M10 (cytochrome P450) haplotypes in southern house mosquito and its possible implication for vector control

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Overexpressions of a cytochrome P450 gene, CYP9M10, have been shown to associate with pyrethroid resistance in several independent strains of the southern house mosquito Culex quinquefasciatus. There are two forms of overproducing CYP9M10 haplotypes sharing an insertion of the same transposable element, while one is duplicated (D-Cu(+) form) and the other is not duplicated (Cu(+) form). The D-Cu(+) form was apparently derived from a haplotype of the Cu(+) form through a recent gene duplication after the TE insertion. The D-Cu(+) haplotype showed higher overexpression and was associated with stronger resistance compared to the Cu(+) haplotype, suggesting that the D-Cu(+) haplotype has acquired stronger efficacy to resistance by gene duplication and/or other cis-acting regulatory mutation(s). CYP9M10 haplotypes with the Cu(+) and D-Cu(+) forms have been found from strain or samples originating from geographically distinct areas including Asia, Middle East and Africa suggesting worldwide distribution of these haplotypes. On the other hand, the nucleotide sequence among the Cu(+) and D-Cu(+) haplotypes were highly identical regardless of place they were isolated indicating that they share a very recent common ancestral haplotype. Although the Cu(+) and D-Cu(+) CYP9M10 haplotypes are considered to spread under insecticide selection, they are not considered as an immediate threat to the actual control practices so far because the resistances associated with these haplotypes have been confirmed larval specific while pyrethroids are mainly used to combat adult mosquitoes. However, some attention may be required to see whether there is another regulatory variant of CYP9M10 overexpressed even in adult stage.

Keywords:*cytochrome P450, insecticide resistance*

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Proliferation of pyrethroid-resistant bed bugs in Japan

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The bed bugs are reemerging pest in Japan. The infestations of bed bug are increasing from the second half of the 2000s. The one important factor seems pyrethroid resistance. The bed bugs highly resistant to pyrethroids were already found in U.S. and elsewhere. It was probable that same situation in the pyrethroid resistance as U.S. in Japan. Recent studies have shown that amino acid mutations in sodium channel (VSSC) associated with pyrethroid resistance in bed bug, Cimex lectularius. Sixty seven colonies (total: n=461) of bed bug were collected from various locations in Japan from 2009 to 2011. The frequencies of two mutations (V924L and L925I) in the sodium channel gene were investigated in the bed bug colonies. Among the 67 colonies, only single mutation (L925I) found in the 54 colonies, only double mutation(V924L and L925I) found in the 6 colonies. One colony consisted of individuals with both the single and the double mutation. No mutation was found in the remaining 7 colonies. I925 common in two haplotypes was found at broad area in Japan and the frequency was ca.90%. The almost bed bugs were homozygous for I925. The living bed bugs in collected were assayed for pyrethroid resistance. The bioassays showed that the almost bed bugs were pyrethroid resistant, which had I925 mutation(s). Pyrethroids are less effective in bed bug control in Japan.

Keywords:*Cimex lectularius, sodium channel, pyrethroid resistance, kdr*

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Molecular mechanism and detection of pyrethroid resistance in the common bed bug, *Cimex lectularius*

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The common bed bug, Cimex lectularius L. (Hemiptera: Cimicidae), is an ectoparasitic pest that feeds on humans as well as other mammals. We investigate that point mutations on the voltage-sensitive sodium channel are associated with the resistance to pyrethroids. Two point mutations (V419L and L925I) in the voltage-sensitive sodium channel (VSSC) α-subunit gene have been identified in deltamethrin-resistant bed bugs. L925I, located the intracellular loop between IIS4 and IIS5, has been previously found in a highly pyrethroid-resistant populations of whitefly. V419L, located in the IS6 transmembrane segment, is a novel mutation. To establish a population-based genotyping method as a molecular resistance monitoring tool, a quantitative sequencing (QS) protocol was developed. Frequency prediction equations were generated from the plots by linear regression, and the signal ratios were shown to highly correlate with resistance allele frequencies ($r2 > 0.993$). In addition to QS, the filter contact vial bioassay (FCVB) method was established and used to determine the baseline susceptibility and resistance of bed bugs to pyrethroids. A pyrethroid-resistant strain showed > 9375- and 6990-fold resistance to deltamethrin and ?-cyhalothrin, respectively. Resistance allele frequencies in different bed bug populations predicted by QS correlated well with the FCVB results, confirming the roles of the two mutations in pyrethroid resistance. Taken together, employment of QS in conjunction with FCVB method should greatly facilitate the detection and monitoring of pyrethroid resistant bed bugs in the field.

Keywords:*Bed bug, Pyrethroid, Resistance, Point mutation, Quatitative sequencing*

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Transcriptional analysis of development and detoxification in *Culex pipiens quinquefasciatus*

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The genomes of three major mosquito vectors of human diseases have been previously sequenced. However, unlike Anopheles gambiae and Aedes aegypti, which have large amounts of gene expression data, Culex pipiens quinquefasciatus has limited transcriptomic resources. In the present study, cytochrome P450 monooxygenases, glutathione S-transferases, and carboxylcholinesterases of C. p. quinquefasciatu were analyzed for phylogenetic classification. Compared with three other dipteran species, gene expansion in Culex mainly occurred in the genes of α-esterases, juvenile hormone esterases, and P450 CYP325s. Gene expression during various developmental stages and the differential expression responsible for parathion resistance were profiled using the digital gene expression (DGE) technique. A total of 13314 genes were expressed in five DGE libraries. Genes with signal transduction and odorant binding functions were prominently expressed during egg development. Genes involved in proteolysis, glycosphingolipid biosynthesis, and purine metabolism were preferentially expressed at the larval stage. One fourth of the carboxylcholinesterase and P450 genes were expressed at unique stages. Fifteen detoxification genes were expressed at higher levels in a parathion-resistant strain than in a susceptible strain. The results of the present study provide new insights into the functions and evolution of three detoxification gene families and comprehensive transcriptomic resources for C. p. quinquefasciatus.

Keywords:*carboxylcholinesterases, cytochrome P450 monooxygenases, glutathione S-transferases*

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Management of head louse resistance using molecular tools

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Head lice resistance to permethrin is mainly conferred by the knockdown resistance (kdr), a voltage-sensitive sodium channel (VSSC) insensitivity factor. Three VSSC mutations (M815I, T917I and L920F) have been identified. Functional analysis of the mutations using the house fly VSSC expressed in Xenopus oocytes revealed that the permethrin sensitivity is reduced by the M827I (M815I) and L932F (L920F) mutations when expressed alone but virtually abolished by the T929I (T917I) mutation, either alone or in combination. Thus, the T917I mutation is primarily responsible for permethrin resistance in head lice. Comparison of the expression rates of channel variants indicates that the M815I mutation may play a role in rescuing the decreased expression of channels containing T917I. A step-wise resistance monitoring system has been established based on molecular resistance detection techniques. Quantitative sequencing (QS) has been developed to predict the VSSC mutation frequency at a population basis. The speed, simplicity and accuracy of QS made it an ideal candidate for a routine primary resistance monitoring tool to screen a large number of wild populations as an alternative to conventional bioassay. As a secondary monitoring method, real-time PASA (rtPASA) has been devised for more precise determination of low resistance allele frequencies. To obtain more detailed information on resistance allele zygosity, as well as allele frequency, serial invasive signal amplification reaction (SISAR) has been developed as an individual genotyping method. Our approach of using three tiers of molecular resistance detection should facilitate large-scale routine resistance monitoring of permethrin resistance in head lice using field-collected samples.

Keywords:*Head lice, Resistance, Monitoring, Molecular tools*

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Molecular evidence of *Bartonella quintana* in *Pediculus humanus capitis* (Pediculidae: Anoplura) infesting young people in selected villages in Laguna province, Philippines

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Bartonella quintana is a bacterium that multiplies in the gut of the body louse (Pediculus humanus humanus) causing trench fever in humans. B. quintana has been found in body lice of homeless, alcoholic and immunocompromised persons from cities in Europe, USA and Japan. Sasaki and associates (2002) found that body lice from 16.7% (2/12) homeless persons were positive for B. quintana by PCR. They showed that head lice (P.h. capitis) may be involved in the transmission of B. quintana in Nepal. Pediculosis by head lice is a major public health problem among children in the Philippines. This study determined the incidence of B. quintana in head lice collected from young people in selected villages in the province of Laguna, Philippines. Head lice were collected using fine-toothed comb, pooled by host (e.g. 1-10 head lice/pool) and stored at -80ºC. Fifty pools were homogenized and processed for DNA extraction using the protocols of Mini Kit (QIAGEN, USA) and the Blood and Body Fluid Spin. Gene purification and DNA sequencing were also conducted. PCR based detection using Bartonella genus specific primers demonstrated the presence of B. quintana in 2% (1/50) head lice pools. This is the first molecular evidence of the incidence of B. quintana in head lice in the Philippines. Further investigation is needed to determine the prevalence of B. quintana in head lice in the country. The information is useful for the national government to plan and implement interventions, health ordinances and advocacy on pediculosis and Bartonella infections in the Philippines.

Keywords:*Bartonella quintana, head lice, trench fever*

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Diversity of *Bartonella* spp. in flea vectors - insights from studies in Israel

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Bartonellae are small gram-negative bacteria belonging to the alpha-2 subdivision of the Proteobacteria. They infect mammalian erythrocytes and endothelial cells and are transmitted by blood-feeding arthropods. Rodents were found to have a high prevalence of Bartonella infection worldwide, with high diversity of Bartonella spp. and strains. The close contact between human and rodent populations creates permissive conditions for transmission of Bartonella spp. from rodents to humans. A recent survey carried out in the Tel Aviv region, the biggest urban center in Israel, demonstrated the occurrence of a single Bartonella strain closely related to B. elizabethae and B. tribocorum in commensal rats (Rattus rattus). Another study carried out in the Negev desert demonstrated sixteen different Bartonella genotypes in flea pools collected from 5 different wild rodents (jirds, gerbils and mice), indicating the great diversity of Bartonella spp. in rodent fleas, and their capacity to harbor several Bartonella genotypes. Bartonella henselae was detected in three flea-pools indicating the public health importance of rodent-related Bartonella. A recent experimental study have shown that the rodent flea, Xenopsylla ramesis, can acquire several Bartonella genotypes from different wild rodents, and can remain persistently infected for prolonged periods, suggesting their potential capability to serve as competent vectors for Bartonella strains. Additional experimental study showed that X. ramesis is a competent vector of Bartonella OE 1-1. The observed differences in Bartonella prevalence and diversity among rodents in urban versus rural regions request further investigation. The finding of zoonotic Bartonella spp. in rodent fleas has public health importance.

Keywords:*Bartonella, Fleas, Rodents*

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Bartonella species in rodent fleas in Japan

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Accumulating studies have shown that Bartonella species are widely prevalent among wild rodents at high rate. Effective transmission of the organisms is thought to be indispensable to maintain the high prevalence of Bartonella in natural environments. The purpose of this study is to assess the roles of flea infestation for the transmission of the organisms among wild rodent in Japan. Blood and flea (32 Ctenophthalmus congener truncus, 5 Neopsylla sasai, and 3 Hystrichopsylla microti) samples were collected from wild rodents (31 Apodemus speciosus, 5 A. argenteus, and 17 Myodes rufocanus bedfordiae), which were captured in Hokkaido, Japan and examined for Bartonella infection. Bartonellae was isolated from 36 of 53 (67.9%) animals and the DNA was detected from 16 of 40 (40.0%) flea samples. Sequence analysis demonstrated that 4 of 16 (25%) of Bartonella -positive animals were infested with the flea from which the closely related Bartonella DNA was detected, indicating the possibility that fleas acquired Bartonella from the infested rodents. It was indicated that host-parasite specificity may have been established between Apodemus mice and B. grahamii and that the transmission may be accomplished by fleas, N. sasai and/or C. congener truncus by comparing the DNA of Bartonella found in each rodent and its fleas. The efficacy of Bartonella transmission may differ depending on the combination of rodent and flea species. Efficient transmission by favorable vectors in wild rodents and optimal host-parasite interactions could contribute to the high prevalence and long-term infection of Bartonella species.

Keywords:*Bartonella, flea, rodent, transmission, zoonosis*

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S1308F01

Medical & Veterinary Entomology

S1308

Ecology of malaria vectors and their *Plasmodium* parasites in Malaysia

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The four species of Plasmodium affecting humans and their vectors have been well established. However, in Southeast Asia the fifth malaria parasite, P. knowlesi is affecting humans in areas where long-tailed macaques and suitable Anopheles vectors co-exist. With deforestation and changing ecological landscape, simian malaria vectors are also feeding on humans thus transmitting the parasites to them. Two established vectors, Anopheles latens and Anopheles cracens are simio-anthropophagic and acrodendrophilic, thus are excellent vectors of simian malaria. These mosquitoes belong to the An. leucosphyrus group which has limited distribution in Southeast Asia. Studies are currently ongoing to determine the other vectors which keep the transmission on going in the non-human primates. Since the country has embarked on a malaria elimination programme, it is important to know the vectors that have colonized new areas due to changing ecological landscape.

Keywords:*changing environment, malaria vector,*

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Medical & Veterinary Entomology

S1308

Surveillance of malaria vectors and *Plasmodium vivax* in Korea

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Plasmodium vivax was endemic on the Korean Peninsulae for many centuries until the late 1970's. Malaria re-emerged among ROK military populations along the demilitarized zone (DMZ) in 1993 and rapidly spread to nearby civilian communities. By 2000, the total number of reported cases increased to 4,142. From 2000 through 2004, the number of malaria cases rapidly declined to 826, most likely as a result of increased awareness, vigilance, and an expanded ROK military chemoprophylaxis program that included 160,000 soldiers. While it was presumed that malaria would rapidly expand throughout Korea, nearly all transmission remained near the DMZ. As new information was discovered, including two previously unrecognized species (An. kleini and An. belenrae), geospatial distributions (high populations of An. kleini populations near the DMZ with low populations south of Seoul), and vector potential, the identification of malaria health threat risks became more apparent. With the integration of air-conditioned barracks from ill-kept tents, malaria cases among US military decreased dramatically. In addition to preliminary evidence, which demonstrates that civilian communities serve as reservoirs for vivax malaria and infections among ROK military, high infection rates among mosquitoes were observed in mosquitoes collected in the DMZ. These data indicate that the malaria situation is much worse in North Korea than in South Korea. Comprehensive mosquito-borne disease surveillance programs are required to better understand the dynamics of the epidemiology of malaria in Korea and to identify present and future health risks.

Keywords:*Republic of Korea, Anopheles, Malaria*

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Taxonomic status and distribution of *Anopheles Hyrcanus* Group vectors of malaria in Asia

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Anopheles Hyrcanus Group includes several species which are vectors of malaria and other mosquito borne diseases in the Oriental and Palearctic Regions. The group comprises 30 known species world-wide, representing about 42% of the species in the Myzorhynchus Series of genus Anopheles Meigen subgenus Anopheles Meigen. Twenty eight of these species are found in the Oriental and Eastern Palearctic Regions and three in the Western Palearctic Region. In Asian countries (e.g. China, Korea and Japan), the known or potential vectors of human malaria include An. sinensis Wiedemann, An. kleini Rueda, An. pullus Yamada, An. belenrae Rueda and An. lesteri Baisas and Hu. The Hyrcanus Group-species breed in various habitats, such as rice paddies, irrigation ditches, drainage ditches, ground pools, swamps, stream margins, inlets and pools, uncultivated fields, and artificial containers. Surveys from various Asian countries showed that An. sinensis is the most dominant species from rice paddies, irrigation ditches and other habitats. The taxonomic status, distribution records, and medical importance of several Asian species are noted and discussed.

Keywords:*Culicidae, Diptera, Anopheles, Hyrcanus Group, malaria, mosquitoes*

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VectorMap, an online mapping resource for malaria vectors and related groups in Asia and Africa

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VectorMap (www.vectormap.org) is a free resource for mapping collection data and distribution models of arthropods (mosquitoes, ticks, sand flies) involved in disease transmission. VectorMap is an outgrowth of MosquitoMap that currently hosts around 250,000 mosquito records, and over 45 ecological niche distribution models for Asian and African anopheline mosquito species. Collection data are comprised of vouchered specimen information, and both unpublished and unpublished observation data, and are quality controlled for geographic and taxonomic information. Users can view the locations of past malaria vector collections, the results of models that predict the geographic extent of individual vector species, and various malaria distribution layers, all in a GIS-type setting. Collection records are searchable and downloadable, and collection forms, and Excel mapping tools, are available for data contributors to compile and map their data. Two enhancements to VectorMap are under development ? the Mal-area Calculator (MAC) and VectorSurv. The MAC allows the quantification, for any area of interest, of the overlap of vectors, hosts, and disease, to generate an estimate of hazard and risk. The Mal-area approach emphasizes the independent contribution to disease risk of the vector species predicted present. VectorSurv is designed for surveillance data and allows the user to understand when vector population densities are above average, by providing information-rich graphs of species seasonality and abundance. These resources are offered to the international malaria vector control community as a focal point for publishing vector collection data, and for exploring the nexus between geography and malaria transmission.

Keywords:*GIS, Malaria*

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Medical & Veterinary Entomology

Influence of pyriproxyfen on the emergence and survival of malaria vectors in Thailand

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In the current study, we evaluated the efficacy of pyriproxyfen on the emergence and survival of two important malaria vectors (Anopheles minimus [laboratory strain] and An. epiroticus [field strain]) under the laboratory and semi-field conditions. Anopheles minimus has been incriminated as a vector of malaria in Thailand. It is commonly found throughout the country along shaded areas with slow running streams; in forests associated with cleared foothills that are the margin of villages. Anopheles epiroticus is considered to be the malaria vector in coastal areas in Thailand. It breeds mainly in man-made brackish water containers (eg. old fish fermented cement tanks, tanks for shrimp culture). The residual effect of pyriproxyfen for Anopheles control was evaluated for 12 weeks. Moreover, the pyriproxyfen horizontal transfer from the pyriproxyfen-treated surface to the breeding containers by An. minimus and An. epiroticus was conducted in large mosquito cages.

Keywords:*Malaria Vectors*

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Molecular identification and population structure of the malaria vector, *Anopheles sinensis* (Diptera: Culicidae) in China

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Anopheles sinensis is a competent malaria vector in China. It is difficult to distinguish by morphology because of some sibling species. And there is no adequate data of A. sinensis population genetics available yet. The AS-PCR assay for distinguishing A. sinensis from Hyrcanus group was established. The species-specific primers were designed by rDNA-ITS2 sequences differences. The length polymorphism of PCR products can identify the A. sinensis, A. pullus and A. lesteri. Five microsatellite loci were used to estimate population genetic diversity, genetic differentiation and demographic history of A. sinensis from 14 representative localities in China. All 5 loci were highly polymorphic across populations, with high allelic richness and heterozygosity. Hardy-Weinberg disequilibrium was found in 12 populations associated with heterozygote deficits, which was likely caused by the presence of null allele and the Wahlund effect. Bayesian clustering analysis revealed two gene pools, grouping samples into two population clusters; one includes six and the other includes eight populations. Out of 14 samples, six samples were mixed with individuals from both gene pools, indicating the coexistence of two genetic units in the areas sampled. The overall differentiation between two genetic pools was moderate (FST=0.156). No evidence of isolation by distance was detected among populations neither within nor between the two clusters. Variable effective population size and other demographic effects of historical population perturbations could be the factors affecting the population differentiation. The structured populations may limit the migration of genes under pressures/selections, such as insecticides and immune genes against malaria.

Keywords:*Anopheles sinensis, molecular identification, population structure*

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Malaria vector control in western Kenya

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Insecticide treated bed nets (ITNs) have been used to control malaria vectors in Kenya. However, malaria transmission remains high among residents in the Lake Victoria region. It is reasonable to assume that the environment associated with the lake still maintains a high number of malaria vectors. To reveal the factors affecting the high transmission, we monitored use and coverage of bed nets, vector abundance, malaria prevalence among children and related environmental factors for five years. Then, we examined their relationships and evaluated the effectiveness of ITNs. We found that rainfall and lake water level were important factors affecting vector abundance and transmission. The recent intensified climate variation was partially responsible for the patterns and amount of rainfall and lake fluctuation. Although ITNs were effective, the use and coverage of ITNs started decreasing within two years after the mass distribution. The ITN use was particularly low among school children and their prevalence was high. We found the limitation of ITNs, and additional vector control measures are necessary for a considerable reduction of malaria transmission in the region.

Keywords:*malaria, Anopheles, Africa, mosquito, bed net*

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Geographical distribution of *Anopheles gambiae* and *An. arabiensis* in Kenya.

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Since major African malaria vectors, Anopheles gambiae and An. arabiensis have different vectorial capacities, understanding their geographical distributions and relative abundance is important for malaria control. Although the distributions of these species in Kenya had been reported, the available information was old and leaned on a few specific areas. We collected the An. gambiae complex larvae from about 500 sites in Kenya from 2008 to 2010. Our research revealed that 1) An. arabiensis distributed throughout Kenya while An. gambiae distributed mainly in the western part, 2) An. arabiensis also occurred in high altitude areas including Nairobi where malaria vectors had not been recorded for decades, and 3) the relative abundance of An. arabiensis remained high even in low malaria transmission areas. Further we predicted the suitable habitat range of each species using maximum entropy niche modeling with environmental covariates of each collection sites. Environmental covariates included a) mean monthly minimum and maximum temperatures, b) mean monthly precipitation, c) elevation and d) land cover type. The best model was selected based on AUC. The selected model revealed that dry season precipitation is the most important factor of presence of each species. This study added several new distributional records of malaria mosquitoes in Kenya and new information on the environmental factors affecting mosquito distribution.

Keywords:*Anopheles gambiae, An. arabiensis, Kenya, MaxEnt*

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Multimodal pyrethroid resistance in malaria vectors in western Kenya

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In Kenya, a large number of ITNs have been distributed through programs of MOH and NGOs, resulting in a significant reduction in malaria deaths. Since then, however, a high level of pyrethroid resistance caused by the high frequency of point mutations (L1014S) have been detected in An. gambiae s.s. and P450-related pyrethroid resistance seemed to be widespread in both An. arabiensis and An. funestus s.s. A lack of cross-resistance between DDT and permethrin was also found in An. arabiensis and An. funestus s.s., while An. gambiae s.s. was resistant to both insecticides. It is noteworthy that that 2 different resistance mechanisms have developed in the 2 sibling species in the same area individually. The cross resistance between permethrin and DDT in An. gambiae s.s. may be attributed to the high frequency of kdr mutation, which might be selected by the frequent exposure to ITNs. Similarly, the metabolic pyrethroid resistance in An. arabiensis and An. funestus s.s. is thought to develop without strong selection by DDT. The allelic frequency of L1014S was higher in An. gambiae s.s. and An. arabiensis in western regions, although the allelic frequency in An. arabiensis was quite lower than in An. gambiae s.s. The present contribution of the L1014S mutation to pyrethroid resistance in An. arabiensis may be negligible. However, the homozygous frequency could increase with continuing selection pressure due to expanded LLIN coverage in the future. The possible use and mode of action of LLIN against the above pyrethroid-resistant malaria vectors will also be discussed.

Keywords:*malaria, control, pyrethroid, resistance, kdr, Anopheles gambiae, Anopheles funestus*

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Molecular identification of *Anopheles gambiae* complex species (Diptera: Culicidae) and its molecular forms in Liberia

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The Anopheles gambiae complex includes a group of 5 morphologically indistinguishable species which represent the main malaria vector in Sub-Saharan Africa. We collected mosquitoes throughout 3 counties in Liberia from May, July, September and November 2011 using 5 trapping techniques. Molecular techniques were used to identify the Anopheles complex using the differences of rDNA region (28S coding region and 5` end of IGS region) between species and the molecular forms (S and M) of An. gambiae s.s specimens. Over 1500 An. gambiae specimens were collected and identified. For each specimen, legs were excised,DNA extracted and a multiplex PCR was performed using specific primers for each species. After PCR, RFLP (Restriction Fragment Length Polymorphism) method was performed using HahI restriction enzyme. This enzyme digests specific restriction site located at rDNA region of An. gambiae S form gave 2 fragments, due to the absence of this restriction site in M form it remains as a one single fragment. Preliminary results show the widespread of the An. gambiae s.s in Liberia collection sites; only one An. melas/An.merus was detected. We found 77% of An.gambiae specimens were S form while 22% were M form. Only 1% of specimens was determined to be hybrid M/S.

Keywords:*An. gambiae complex*

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Ecological and evolutionary determinants of host species choice in African malaria vectors

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Here we conducted for the first time an experimental investigation of the strength of selection acting on African malaria vectors An. arabiensis and An. gambiae s.s to preferentially specialize on human instead of animal hosts within natural transmission settings. We used novel semi-field mosquito biosphere within a malaria endemic region of Africa to directly investigate the strength of counterselection placed on vector host species choice by the control measures commonly used in the field: bednets. We experimentally measured the feeding success and subsequent fitness of the two major African vectors (> 16,000 mosquitoes) on humans and other potential host types they co-occur. Experimental data were used to parameterize a stochastic life ? history model that combined all measured impacts of host species choice on vector fitness to predict the cumulative effects on the key metric of selection: total lifetime reproductive success. We demonstrated that mosquito fitness depends on host species choice, and the use of bednets substantially reduced the fitness ranking of humans relative to other animal alternatives. For An. arabiensis, the vector now dominating in many parts of Africa, the use of bednet widened the fitness differential between humans and cows to an extent where strong selection for zoophily is expected when most people use nets, and cattle are available. This provides baseline evidence that evolutionary sustainable control approaches incorporating the use of selection for mosquitoes to shift their host choice could enhance the effectiveness of frontline disease control strategies

Keywords:*mosquito vector, anopheles arabiensis, fitness, host species choice, ecology, behaviour*

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Assessment of perceptions of community users and bioefficacy of permethrin incorporated OlysetTM net (LLIN) distributed 5 years before in India

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In Indian context it is important to evaluate long-term field performance of long lasting insecticidal nets (LLIN), especially the reduction in malarial cases and assessment of community acceptance. Hence we carried out community based survey in a District GautamBudh Nagar, U.P, where Olyset nets were distributed 5 years ago, to assess the efficacy of Olyset nets on malarial cases and safety related to it. Three villages were selected for trial and baseline entomological and epidemiological data was collected for one year. After one-year OlysetTM nets were distributed in one village, untreated nets were distributed in another village and third village was kept as control where nets were not distributed. A survey of OlysetTM nets was conducted to examine the perception of community members regarding durability, wash resistance, and comfort of use. Bioefficacy of field used nets was determined by bioassays against malaria vectors and comparing the malariometric indices before distribution and during 5th year of Olyset nets use. Use of OlysetTM nets reduced the malarial cases drastically. Bioefficacy studies proved that Olyset nets fulfill the criteria of LLIN. People liked the size, colour of these nets and appreciated nets in relation to the efficacy against mosquitoes and preferred their larger mesh size as it permits more airflow. OlysetTM nets fulfill the LLIN criteria and show durability with much longer insecticide persistence even after 5 years of use. Even epidemiological data have confirmed that these nets are highly effective and proved to be a good physical barrier between man? vector contacts.

Keywords:*LLIN, Olyset, malariometric indices, Bioefficacy*

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Can olfactory memory alter oviposition choice in malaria vectors?

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Oviposition site selection in mosquitoes is mediated by physical and chemical factors present in the aquatic habitats. Various organic materials and bacteria have been associated with this process. Though mosquitoes are innately attracted to or repelled by certain compounds, the chemical character of potential oviposition sites change over space and time. Mosquitoes might respond to this variability by altering their behaviour based on prior experience as a larva. Olfactory memory and its impact on oviposition choice has been described for Aedes and Culex species but has not yet been explored in anophelines. If the oviposition behaviour is inherited and defined in Anopheles mosquitoes, this means that the chemicals involved in this process can be identified, manipulated and used in 'attract & kill' or 'push-pull' strategies to improve malaria vector control. To investigate olfactory memory in oviposition by Anopheles gambiae, larvae were reared in grass infusion previously avoided by gravid adults in open field habitats. The resulting adults were offered grass infusion and tap water in two-choice cage bioassays. Preferences were compared to mosquitoes reared in tap water. Mosquitoes that were conditioned in grass infusion and those reared in tap water preferred to lay eggs in tap water. The repellent effect of the grass infusion cannot be altered by rearing the vector in it. In contrast to some Culex and Aedes species the ability of An. gambiae to select oviposition sites appears not to be affected by the experience of inmature stages.

Keywords:*Anopheles, mosquitoes, oviposition, malaria, olfactory memory, vector control*

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A novel approach of evaluating and analyzing oviposition bioassays for malaria mosquitoes

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As physiological resistance to insecticides and behavioural avoidance of interventions continue to defy costly frontline mosquito management strategies in Africa, the need to develop and integrate sustainable and effective solutions for dealing with these errant vectors of malaria remains pressing. A good understanding of the process of host seeking in Anopheles gambiae s.l. has instigated many effective intervention strategies. In much the same way, new insight on the little defined return journey when gravid mosquitoes leave their resting places after successful blood meals and lay eggs in select aquatic breeding sites could inform new fronts in management of these vectors. We looked into the periodicity of oviposition together with egg number and distribution as well as the impact of different host blood meal sources with the object of developing sound systems for studying the process and cues of oviposition site selection in Anopheles gambiae s.l. The choice of host is demonstrated to influence the proportions of caged An. gambiae s.s. that become gravid following blood meals in favour their natural host, humans. Individual mosquitoes showed a wide variability in numbers of eggs laid and spread these across more than one substrate making it difficult to quantify its preferences for oviposition. Caged gravid mosquitoes consistently laid eggs in early scotophase with over 90% laying eggs between 17:00 and 21:00hrs. We present a novel way of implementing and analysing data from oviposition bioassays for An. gambiae s.l.

Keywords:*Anopheles, Oviposition, Malaria, Mosquitoes, Vector management*

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Medical & Veterinary Entomology

Biological and behavioral characterization of *Anopheles funestus*-like species from Northern Malawi

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Introduction Malaria in Malawi is transmitted by An. gambiae, An. arabiensis and An. funestus. This vector diversity is further complicated by the recently discovered new An. funestus-like species which is morphologically similar to An. funestus, and found in association with humans. This study investigated the biology and behavior of the An. funestus-like species in relation to malaria transmission. Materials and Methods Mosquitoes were collected resting indoors and outdoors from study sites. Specimens were identified morphologically and by PCR as An. funestus-like species. An. funestus-like were analyzed for blood meal source and Plasmodium sporozoite infection using ELISA. Results In total, 392 adult An. funestus group mosquitoes were collected during April and September, 2010. Karonga contributed for 63.8% and Likoma Island 36.2%. Of these, An. funestus-like comprised 8.7% (34/392). Most of the An. funestus-like species were collected resting indoors 91.2% (31/34) compared to outdoors 8.8% (3/34). The species was predominant in the dry season 64.7% (22/34). Nineteen An. funestus-like females were analyzed for blood meal source. Goat blood was found in 10 specimens, of which 7 had also taken cow blood. An overall dry season infection rate of An. funestus-like species by Plasmodium vivax was 5% (1/20) in this study and 3.1% (2/64) from a 2009 analysis. However, false-positivity still needs to be excluded. Discussion and conclusion This study confirmed An. funestus-like species occurring in sympatry with An. funestus group members. An. funestus-like species was predominantly found resting indoors but preferring animal over human blood. Its vectorial capacity needs confirmation

Keywords:*An. funestus-like, indoor resting, zoophilic*

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Invasive species, conservation biology, and biological control - a three way collision: Now time to join forces

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Invasive species may strongly affect native plants and in doing so, affect biodiversity at both the plant level and at the level of the specialized insects that depend on the plants. Biological control may be used to suppress densities of such invasive pests and often represent the only practical, permanent and widespread solution to such problems. Here we examine importance of invasive species as threats to native insect biodiversity, relative to other categories of threats and the potential of biological control to contribute to the restoration of native species or ecosystems damaged by invasive organisms. We focus on two invasive insects, Icerya purchasi Maskell (Hemiptera: Monophlebidae) in the Galapagos Islands and Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) in the ash forests of the north central United States. The application of biological control is not embraced fully by the conservation biology and ecological restoration community, in part for lack of familiarity, and in part because of the perception that introduced natural enemies may themselves become damaging to native biodiversity. We argue that ecosystem restoration following invasions by highly damaging species requires use of biological control within a conservation biology framework with the active joint efforts of both biological control scientists and conservation biologists. Efforts to promote this approach will be discussed.

Keywords:*Invasive species, conservation biology, biological control*

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Ecological impacts of red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) on ant community in China

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Red imported fire ant (RIFA) Solenopsis invicta Buren is hazardous alien species, which had been found in Guangdong, China on Sept., 28, 2004. The impact of the fire ant invasion on ecosystem was thought to be significant, and cause the decrease of the biodiversity in the previous reports. RIFA also often competed with native ants and replaced them finally. As a new invasive species in south China, effect of the fire ant on the biodiversity, especially the ants community with the similar niche needs to be known further. Therefore, the effects of RIFA invasion on the diversity of ant community in the orchard, wasteland and lawn in Guangdong were studied by the method of the bait trap and pitfall trap in this study. The competition between RIFA and native ants was also observed.

Keywords:*invasion, impact, Solenopsis invicta, ant community, diversity, interspecific competition*

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Impacts of two alien ants, *Anoplolepis gracilipes* and *Pheidole megacephala*, on native ant fauna in Okinawa, Japan

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Exclusion of native species is one of the most serious impacts caused by invasive alien species. Anoplolepis gracilipes (yellow crazy ant: YCA) and Pheidole megacephala (bigheaded ant: BHA) are widely known as invasive ant species due to their negative impacts on ecosystems in the world. However, even though they have been reported as alien species in Okinawa main land, their impacts on native ant fauna are unknown. Ant fauna was investigated at sites infested by YCA or BHA and non-infested sites. Abundance of each of all ant species and species richness were compared between them. For both of the two alien species, ant fauna differed significantly between infested and non-infested sites (ANOSIM, $p<0.01$). However, the effects differed greatly between the two species. YCA did not have negative impacts on abundances of native species nor species richness. Tetramorium kraepelini and Monomorium chinense increased in the infested sites (GLMM, $p<0.05$). In contrast, BHA had strong negative impacts on native ant fauna. Abundances of three major species, Tetramorium bicarinatum, Monomorium chinense, and Pheidole fervens, and species richness decreased in the infested sites (GLMM, $p<0.05$). However, in our survey, BHA disappeared from five sites out of seven, in which BHA was distributed in the preliminary survey. This result indicates that BHA colony does not persist for long though the reasons remain unclear. Thus, in Okinawa, the impacts of invasion by YCA and BHA on native ant fauna are relatively small in comparison to previous studies conducted in abroad.

Keywords:*invasive species, Anoplolepis gracilipes, Pheidole megacephala*

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Understanding invasion history and the eradication project of the Argentine ant in Japan

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The Argentine ant, Linepithema humile, has successfully spread from its native range in South America across much of the globe. A form of social structure, supercoloniality, whereby individuals from separate nests cooperate, is attributed to its successful worldwide expansion. The Argentine ant, first noted in 1993, is now found in several regions of Japan. Therefore, early detection as well as rapid control is required to prevent further expansion of the species. We attempted to demonstrate the genetic structure of introduced Argentine ant populations in Japan and how they relate to other introduced and native populations to understand its invasion history. Furthermore, we have demonstrated the eradication project of Argentine ant in Tokyo to provide successful example for the support the ongoing battle against Argentine ant.

Keywords:*Invasive species, Management and eradication, Social insect*

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Ecological and economic impact of the alien B and Q whiteflies of the *Bemisia tabaci* species complex in China

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The whitefly Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a species complex containing over 30 morphologically indistinguishable species. In the last 20-30 years, two of the putative species, commonly known as the B and Q whiteflies, have invaded many parts of the world. In China, the alien B and Q whiteflies as well as 13 indigenous species have been recorded. Records indicate that the first invasion in China by B occurred around 1994 and that by Q occurred around 2003, and both were probably aided by imports of ornamental plants. By 2003, B had been recorded from 15 provinces, mostly along the south-east costal regions, and by 2009/2010, B had been recorded from 27 provinces and Q from 26 provinces, nearly all over China. Following the invasions, whitefly outbreaks occurred on many field and vegetable crops, and indigenous whitefly species were displaced by the invaders in many regions of invasion. Meanwhile, disease pandemics caused by indigenous begomoviruses, which are exclusively transmitted by whiteflies, started to occur in southeast China around 2000. Following the invasion of the alien Tomato yellow leaf curl virus in 2005-2006, disease pandemics caused by TYLCV and indigenous begomoviruses have been increasing rapidly and widespread, causing enormous yield losses in a number of crops particularly tomato. With their widespread distribution and further invasions into more remote areas, the invasive whiteflies and the begomoviruses they transmit present one of the biggest challenges China agricultural production has to face in the years to come.

Keywords:*Whitefly, begomovirus, biological invasion, invasive species, indigenous species, crop loss*

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The impact of the introduced parasitoid *Torymus sinensis*, a biological control agent of *Dryocosmus kuriphilus*, on the indigenous parasitoid *T. beneficus* on chestnut trees in Japan

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Hybridization between the introduced parasitoid Torymus sinensis and the indigenous congener T. beneficus had attracted attention since T. sinensis was first introduced to control the invasive pest Dryocosmus kuriphilus in Japanese chestnut orchards; researchers were concerned that the hybridization might interfere with the biological pest control by the introduced parasitoid. Such apprehension disappeared as the damage caused by D. kuriphilus decreased drastically. However, morphologically intermediate individuals between the two Torymus species had appeared in the field after the release of T. sinensis. Thus, there has been renewal of interest in the hybridization, e.g. the non-target effect of biological control, in recent years. I have used molecular markers for hybridization surveys in the field, not only because T. sinensis and T. beneficus are similar morphologically, but also because there are two emergence types (early- and late-spring strains) in T. beneficus that cannot be easily discriminated except for the molecular markers. Our surveys show that 1) T. sinensis displaced the two strains of T. beneficus in Japanese chestnut orchards, 2) the hybridization between T. sinensis and early-spring T. beneficus occurred at low frequency (≤ 1%) and that 3), on the other hand, the hybrid F1s between T. sinensis and late-spring T. beneficus were detected at higher frequency (≤ 20%). These results indicate that hybridization played more important role in the displacement of late-spring T. beneficus by T. sinensis than that of early-spring T. beneficus.

Keywords:*Torymus sinensis, Torymus bemeficus, hybridization, displacement, Dryocosmus kuriphilus*

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The invasive bark beetle, *Ips grandicollis*, and climate change: dual risks to bio-control of the woodwasp *Sirex noctilio*

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The woodwasp Sirex noctilio is an exotic pest that can cause extensive losses to pine plantations by introducing its symbiotic fungus Amylostereum areolatum along with a phytotoxic mucus during oviposition. In recent decades a successful bio-control program has operated in Australia using the nematode Beddingia (=Deladenus) siricidicola which feeds upon A. areolatum in pine trees and parasitises S. noctilio causing sterility. Efficiency of this bio-control program is thought to be affected by another exotic pest, the bark beetle Ips grandicollis and its associated symbionts. Ips grandicollis attacks the "trap trees" used to inoculate bio-control nematodes into the S. noctilio population. This may reduce the attractiveness of trap trees to S. noctilio and interfere with B. siricidicola thereby compromising successful bio-control. This paper will report on a major project in which field and laboratory studies have investigated the severity of this problem, interactions between the fungi and nematodes associated with the insects, and possible management strategies. Collectively, the results suggest that climate change and I. grandicollis constitute significant risks to the success of the bio-control program for S. noctilio in Australia.

Keywords:*Invasive pest, climate change, biological control, Amylostereum areolatum, Australia, Beddingia siri*

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Decline of native ladybird beetles caused by an invasive alien predator

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We documented the decline of distribution range (Belgium and Britain) and abundance (Belgium, Britain, Switzerland, Czech Republic) of formerly common and widespread native ladybird beetles (Coleoptera: Coccinellidae) before and after the establishment of Harmonia axyridis that is a globally rapidly expanding invasive alien species. The distribution data were collected mainly from public surveys verified by recognised experts. Most species studied showed substantial declines attributable to H. axyridis. The two spot ladybird, Adalia bipunctata, declined by 30% (Belgium) and 44% (Britain) over five years after the arrival of H. axyridis. The trends in the abundance of ladybird beetles are based on data collated through systematic surveys of deciduous trees in the four countries. These trends revealed similar patterns of declines across three countries. In the Czech Republic, where H. axyridis arrived later, the abundance of many species decreased before the arrival of H. axyridis and thus its contribution to their decline was not significant. The invasive alien species H. axyridis was proved to cause decline in native biodiversity by displacing native ladybirds in Europe, mainly those with high niche overlap, probably through predation and competition for prey.

Keywords:*intraguild predation*

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Reproduction and critical pupation weight in the invasive and native populations *Harmonia axyridis* (Coccinellidae, Coleoptera)

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The Harmonia axyridis (Pallas) imagoes, pupae and larvae were collected at the territory of the Far East and Czech Republic in summer-autumn 2010-2011. In 3-4 days after a molting to IV instars the Harmonia larvae were placed individually without food. Lethality of the H. axyridis as a function of the larvae weight had the threshold character and was described by the S-like curve of logistic regression. The critical pupation weight, at which about 50% of individuals survive, in the invasions Czech population is 26.3 mg, in the Far East population 29.0 mg. It is possible to assume that decrease of the critical pupation weight in the invasions populations is one of those adaptive advantages which have allowed H. axyridis to expand its area. The minimum weight of females in the Czech population is 12.5 mg, in Far East - more than 16.0 mg. The reproduction of these small females corresponded to the mean value for the populations. The average size of an eggs batch is 24-27 eggs that a little more than in Far East populations - 19 eggs. We have not found correlations between the size of an eggs batch and ovarioles number, and also in weight of a female H. axyridis.

Keywords:*Harmonia axyridis, ovarioles number, pupation weight*

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Habitat disturbance disturbance differentially benefits exotic over native coccinellids in agricultural landscape mosaics

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Both anthropogenic habitat disturbance and the breadth of habitat use by exotic species have been found to facilitate invasion into novel environments, and these factors have been hypothesized to be important within coccinellid communities specifically. This study addressed two questions: 1) Do exotic species benefit more than native species from human disturbed habitats? 2) Are exotic species more generalized in their habitat use than natives within the invaded range or can their abundance patterns be explained by specialization on the most common habitats? We quantified the use of different habitat types by native and exotic coccinellid beetles by sampling individuals in nine landcover types that spanned a gradient in disturbance intensity, and represented the dominant natural, semi-natural and agricultural habitats in the landscape. Our results provide strong support for the hypotheses that more disturbed habitats are differentially invaded. Both the proportion of exotic individuals, and the proportion of exotic species increased significantly (4.8 fold and 1.3 fold respectively) with increasing disturbance intensity. In contrast, we found no evidence that exotic species were more generalized in their habitat use than native species, in fact the trend was in the opposite direction. The abundance of exotic specialized coccinellid species was not correlated with the area of the land cover types in the landscape. The results suggest that successful exotic coccinellids may be "disturbance specialists" that thrive within human modified habitats. Therefore, to avoid the expansion of invasive species in agricultural landscapes, less disturbed agricultural production systems could be promoted.

Keywords:*biodiversity, habitat specialization, specialists and generalists, ladybeetles*

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S1401TU11

Invasive Species and Quarantine

The spread of the Asian honeybee, *Apis florea* in Sudan: a test case for invasive insect species

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The spread of the dwarf honeybee, Apis florea, in Sudan along the River Nile in a linear fashion provides a good model to study the population dynamics and genetic analyses of a honeybee invasion. We used microsatellite DNA analyses to asses the population structure of both invasive A. florea and native Apis mellifera along the river. The invasive A. florea had significantly higher population densities than the wild native A. mellifera. Nevertheless, we found no indication of competitive displacement, suggesting that although A. florea has a high invasive potential it coexists with native A. mellifera along the river Nile. On the other hand this high population of A. florea in Sudan appeared to be originated from a single colony introduction more than two decades ago.

Keywords:*Apis florea. Apis mellifera. Microsatellite DNA. Invasion. Competition. River Nile*

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S1402TU01

Invasive Species and Quarantine

Biological attributes contributing to the invasiveness of *Sitona lepidus* in New Zealand and the success of its introduced biological control agent *Microctonus aethiopoides*

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Sitona lepidus Gyllenhal (Coleoptera: Curculionidae) was first reported in New Zealand in 1996 and rapidly became a major pest of white clover (Lolium perenne L.). Half of New Zealand's land area consists of pasture, and this provided S. lepidus with plentiful resources and few natural enemies or competitors. The combination of good flight capability, physical robustness of the adult to withstand and be transported by agricultural machinery and vehicles, and wide climatic tolerance enabled it to spread the length of the country (1,600 km) by 2010. The pattern of invasion demonstrated both natural and assisted dispersal. The species' invasiveness as been facilitated by its flexible life cycle duration, long lived adult stage, and high reproductive rate. In 2006, an Irish biotype of Microctonus aethiopoides Loan (Hymenoptera: Braconidae) was released for biological control of S. lepidus. It has also established and dispersed very rapidly, and often suppresses S. lepidus populations within 2 ? 3 years of its establishment in a new locality. Attributes contributing to the success of the parasitoid include asexual reproduction, density dependent semi-gregarious clutch size (1-4 offspring per host), multiple generations/ year and dispersal of the parasitoid as eggs or early instar larvae within the adult stage of its host. The founding of new populations from as few as ten parasitoids was an important aspect of the biological control release programme in the North Island where the weevil was already widespread before M. aethiopoides was introduced.

Keywords:*Dispersal, clutch size, reproduction, invasion, Sitona lepidus, Microctonus aethiopoides*

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Asymmetric reproductive interference between specialist and generalist predatory *Harmonia* ladybirds

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Invasive generalist predators often displace similar native species through intraguild predation, resource competition, and escape from predation. By contrast, other negative interspecific interactions, such as reproductive interference, are often neglected in the study of invasive generalist predators. In this study, we examined interspecific sexual interactions in two predatory Harmonia ladybirds in their native range in Japan. Harmonia axyridis is a generalist predator, now colonizing the Americas and Europe, whereas H. yedoensis is a rare specialist predator found in Japan that preys only on pine aphids. We showed that mating success substantially decreased in H. yedoensis but not in H. axyridis when adults of the two species were experimentally housed together. Our results indicate that asymmetric reproductive interference from the dominant species may restrict resource use by the non-dominant species. Based on these findings, we discuss the possibility that reproductive interference from invasive generalist predators may affect abundance and resource use of similar native species during biotic invasions.

Keywords:*generalist predator, Harmonia, ladybird, reproductive interference, sibling species*

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S1402TU03

Invasive Species and Quarantine

Evaluating gene flow of the western corn rootworm in a European hybrid zone

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The western corn rootworm Diabrotica virgifera is one of the most serious pests of maize in North America. It is now an invasive pest in Europe. D. virgifera originates in North America and has been introduced several times in various European locations since the 1990's. Two European invasive outbreaks that were initially geographically disconnected are currently in contact and form a hybrid zone in northern Italy. We used this hybrid zone to estimate D. virgifera dispersal by performing an analysis of microsatellite frequency clines (a cline is a continuous variation of allele frequency in space). In absence of selection and given the date of contact between the outbreaks, the slope of the microsatellite frequency clines provides an estimate of gene flow across the hybrid zone. The linkage disequilibrium between the microsatellite loci provides another estimate of gene flow. The cline slope and linkage disequilibrium were estimated by maximum likelihood and provided gene flow estimate with narrow support limits.

Keywords:*clinal analysis, dispersal, microsatellites, maximum likelihood estimation*

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S1402TU04

Invasive Species and Quarantine

Population genetics of interactions *Leptinotarsa decemlineata* and *Coccinella septempunctata*

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Colorado potato beetle (Leptinotarsa decemlineata Say) is invasive species in the Eurasia and the pest of potatoes. He moves slowly through Russia and 35 years ago colonized the Southern Urals. Seven-spot ladybird (Coccinella septempunctata L.) widespread in central Russia, lives in grassy communities, feeds mainly on aphids, and beneficial to agriculture as a biological control agent. Visual observations, the content in the laboratory and the data of PCR - analysis of the gut content of C. septempunctata adults demonstrated they feed on the eggs (possibly small larvae) of the L. decemlineata . Seven-spot ladybirds feeding on the eggs and larvae of Colorado potato beetle differ from ladybirds feeding on the aphids and leads to the development of stress reactions. We conducted a genetic analysis using RAPD - PCR and morphometry to determine the process of adapting population of C. septempunctata to feeding eggs of the L. decemlineata. Analysis of heterozygosity and interpopulation polymorphism C. septempunctata shows the differences between individuals, fed on eggs of Colorado potato beetle and do not fed. Feeding on the invasive species leads to a rapid microevolutionary changes in the population of C. septempunctata. Investigation funded Russian Fund Base Research (grant No 11-04-97022-r_povolzhie_a).

Keywords:*population genetics, Coccinella septempunctata, Leptinotarsa decemlineata, RAPD, morphometry*

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Population genetics of the first plataspid species complex (Hemiptera: Plataspidae) reported in North America

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The state of Georgia, USA, was the Northern Hemisphere entry point for a new Asian insect symbiosis. The insect, Megacopta cribraria, was first reported in October 2009 feeding on kudzu and clinging to the sides of houses in North Georgia by the thousands seeking overwintering shelter under eaves and in attics. Since 2009 the Asian insect has established itself as an urban nuisance and a pest of soybeans and other legumes. Populations have spread rapidly from the state of Georgia into at least eight contiguous U.S. states and into Central America with reports of new infestations coming in almost daily. Insect and endosymbiont-specific polymerase chain reaction (PCR) primers were used to generate gene and genomic sequence data from insect collections across the range of infestation. Analyses of these data revealed a lack of insect maternal diversity and two major endosymbionts: a primary gamma-proteobacterium, a known nutritionally beneficial obligate, and a secondary alpha-proteobacterium, reported to manipulate insect reproduction in favor of females, but may be functioning differently in this Megacopta species. A chi square analysis of 2000 randomly collected bugs showed no significant difference between males and females. Gamma- and alpha-proteobacteria may, therefore, be nutritionally beneficial and thereby contributing to the insect's fitness and awesome invasive power. We present the genetic evidence from the perspective of a Megacopta interspecies complex in order to understand the origin, dispersal and adaptive potential of this insect.

Keywords:*Megacopta cribraria, gamma-proteobacterium, alpha-proteobacterium*

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Cloning, expression and characterization of mitochondrial manganese superoxide dismutase from the Whitefly, *Bemisia tabaci*

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The mitochondrial manganese superoxide dismutase (mMnSOD) is an important metalloenzyme that catalyzes the dismutation of the superoxide anion into hydrogen peroxide. In this study, an mMnSOD was cloned from the invasive Mediterranean cryptic species of the whitefly Bemisia tabaci complex. Sequence analysis indicated the full length cDNA of mMnSOD is of 1,210 bp with a 675 bp open reading frame, corresponding to 224 amino acids with 25 residues of mitochondrial targeting sequence. The predicted protein has a calculated molecular weight of approximately 24.5 kDa and an estimated pI of 8.97. In comparison with the MnSODs of various vertebrate and invertebrate animals, four conserved amino acids for manganese binding (H54, H102, D186 and H190) and MnSOD signature from 186 to 193 (DVWEHAYY) were observed. Recombinant mMnSOD was expressed in Escherichia coli and the enzymatic activity of purified mMnSOD was assayed under various temperatures. In addition, the in vivo activities of MnSOD in the whitefly were measured under various conditions including exposure to low (4°C) and high (40°C) temperatures, transfer from a favorable (cotton) to an unfavorable host plant (tobacco), and treatment with pesticides. We found that the activity of MnSOD is closely related to various stress conditions. The results indicated that the whitefly mMnSOD is involved in the cellular stress response and anti-oxidative processes.

Keywords:*Bemisia tabaci, mitochondrial manganese superoxide dismutase, oxidative stress, whitefly*

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Aggressive interactions between *Solenopsis geminata* and both social forms of *S. invicta* in Taiwan

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Fire ants represent a significant hazard for humans and animals living in endemic areas; they also exhibit agonistic behavior toward non-nestmates. Solenopsis geminata and both social forms of S. invicta were accidentally introduced into Taiwan. In the present study, the differences in aggressive behaviors of three fire ant forms towards conspecifics and heterospecifics were investigated. Workers of all three forms showed significant aggression toward non-nestmate (heterospecifics) in individual assay. In group assay, the mortality of ants were higher for monogyne S. invicta than for polygyne S. invicta after 24 h. These results showing that S. geminata has suffered the most mortality 24 h after inter-specific aggression tests. The outcome of these bioassay may be useful to predict the range expansion and species dominance of three fire ant forms. In addition, we measured the aggressive response of fire ants and to understand their aggressive threshold, potential shifts in populations and spatial distributions.

Keywords:*Solenopsis geminata, S. invicta, aggressive behavior, conspecifics, heterospecifics*

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Little fire ant, *Wasmannia auropunctata*, established on Guam

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Little fire ant (LFA), Wasmannia auropunctata, was detected on Guam in November 2011. Previous infestations in the Pacific Basin include the Hawaiian Islands of Hawaii, Maui and Kauai, New Caledonia, and Northern Queensland, Australia. All of these areas have air and sea connections to Micronesia. The devastating effects of LFA on agriculture and forest ecosystems observed in LFA infested areas are likely to occur on Guam and any other Micronesian island infested by LFA. LFA surveys on Guam suggest that it is presently established on at least seven sites on the island, including a green waste hardfill site of about 2 ha, and several small farms and residential areas. Impacts on invertebrate biodiversity have been noted in infested areas on Guam. The ants' spread to and throughout Guam is most likely due to human transport of infested plant material. Surveillance surveys suggest that it has yet to spread to the neighboring islands of the Commonwealth of the Northern Mariana Islands (CNMI), or elsewhere in Micronesia.

Keywords:*little fire ant, Wasmannia auropunctata, biodiversity, predator*

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S1402TU09

Invasive Species and Quarantine

Introduced Asian paper wasp in New Zealand: Diploid males and triploid females production due to bottleneck *Harmonia axyridis* (Coccinellidae, Coleoptera)

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Asian paper wasp Polistes chinensis is originally distributing around Far East Asia including Japan, Korean peninsula, and east shore of China. The species was accidentally introduced in New Zealand in 1979, and expands its distributing range. I analyzed and compare the population genetic structures between Japan and New Zealand using 10 microsatellite loci and mtDNA haplotypes. The results were as following, (1) although more than 95% of haplotypes were observed in the both countries, the remaining haplotypes was only observed in one of the two countries, (2) the genetic makeup in microsatellite loci in NZ was clearly different from that in Japan, (3) higher frequencies of diploid male and triploid female producing colonies were observed in NZ, suggesting that severe bottleneck operated during the introduction, (4) mean distance between mother and offspring in NZ was twice as large as that in Japan. Taken together, these results suggested that Asian paper wasp in NZ rushes around in North Island after passing through narrow gene flow.

Keywords:*paper wasp, biological invasion, bottleneck, genetic variation*

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The Potential geographical distribution of *Bactrocera correcta* (Diptera: Tephritidae) in China

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The guava fruit fly, Bactrocera correcta (Diptera: Tephritidae), is regulated as an important quarantine pest in China which invaded into Yunnan province recent years. The potential geographical distribution of B. correcta in China was researched using two suitability technical modes of 'CLIMEX + ArcGIS' and 'Suitability experiment + Suitability model + ArcGIS'. With the first mode, 28% areas of China which among the south part from Yangtze River is predicted as potential suitable region under current climate condition, and the suitable region percent will increase to 31% with the suitable range expanding northward to some parts Shandong Peninsula, Shanxi, Shaanxi and Gansu province under future climate condition in 2020, moreover, the suitable range will further increase to 34% with a geographical expanding to Hebei province and Shandong Peninsula in 2050. With the second mode, the eclosion rate model of B. correcta is established and the suitability of B. correcta is predicted with a legible trend from May to September with high degree. According to the eclosion rate model and suitability, 5 monitoring periods are suggested as (1) From Jan. To Dec.: Yunnan, Guangxi, Guangdong and Hainan. (2) From Mar. to Nov.: Sichuan, Guizhou, Hunan, Chongqing, Jiangxi. (3) From Mar. to Oct.: Hubei, Fujian, Taiwan, Shandong, Tibet, Shaanxi, Henan, Anhui, Zhejiang, Jiangsu and Shanghai. (4) From Apr. to Oct.: Xinjiang, Qinghai, Gansu, Shanxi, Hebei, Liaoning, Beijing, Tianjin. (5) From May to Sep.: Ningxia, Heilongjiang, Jilin, Inner Mongolia.

Keywords:*Bactrocera correcta, potential geographical distribution, CLIMEX, ArcGIS, eclosion rate model*

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S1403TH01

Invasive Species and Quarantine

Biological invasions and endosymbiosis in Arthropods, a case study: *Bemisia tabaci*

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Biological invasions are more and more frequent worldwide and understanding this process is a big issue. Invasive success depends on several traits permitting local adaptation. Among those traits, host-plant adaptations or demographic advantages are specific traits involving in some cases endosymbiosis. Intracellular prokaryotic association known as endosymbiosis is a well known-phenomenon which is present at least in 15% of all insects. Among the sap-sucking insects of the suborder Sternorrhyncha, psyllids, whiteflies, aphids, and mealybugs, an obligatory endosymbiont is even crucial to allow them to synthesise essential amino acids. Furthermore, other non obligatory endosymbionts, so-called secondary endosymbionts, are known to be linked to adaptive traits which can enhance invasiveness of their host. These biotic interactions will be discussed through several models and in particular the invasive whitefly Bemisia tabaci. Indeed, two biotypes (B and Q) or "genetic species" of B. tabaci have a tremendous invasive success worldwide since the 80ies. They harbor a wide range of endosymbionts, with potentially 6 secondary endosymbionts (according to biotypes): Cardinium, Arsenophonus, Hamiltonella, Rickettsia, Wolbachia and Fritschea and one obligatory Portiera aleyrodidarum. Recent studies showed significant links between reproductive manipulations (strong female bias, non random hybridization), large fitness benefits, better thermotolerance or transmission efficiency of begomoviruses according to the endosymbionts of their infected hosts. Most of the functions of those secondary endosymbionts are described in other models, and are largely unknown yet in B. tabaci.

Keywords:*invasion, bemisia tabaci*

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Effects of Behavior and Habitat Use on the Spread and Impact of Invasive Herbivores

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One of the most detrimental impacts of invasive species is the exclusion of native species, which reduces biodiversity and alters community structure. However, coexistence between invaders and native species might be promoted by niche partitioning, even when one species is excluded in some habitats. Here, we examined the effects of variation in species traits, behavior, and habitat use on patterns of coexistence or exclusion between invasive and native whitefly species in the Bemisia tabaci species complex. Species in this complex often engage in intense reproductive interference, with males attempting to mate with females of other species. In shared habitats, behavioral traits of invasive whitefly species allow them to more effectively locate and copulate with suitable mates, leading to faster rates of population growth and exclusion of native species. Under field conditions, however, native and invasive whitefly species that exclude each other in shared habitats often coexist. Using spatially-explicit models, we show that niche partitioning can promote coexistence between invasive and native whitefly across broad regions, despite asymmetric reproductive interference and exclusion in some habitats. Combining observational data from a long-term field study in Israel with model results indicated that variation in habitat use was a primary factor driving coexistence between invasive and native whitefly species across landscapes. Our study reveals how models can be linked with empirical data to isolate and test mechanisms underlying patterns of coexistence or exclusion following invasions.

Keywords:*Behavior, Niche partitioning, habitat use, whitefly, reproductive interference, species exclusion*

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Invasion of the horse-chestnut leaf miner in Europe: Implications at all trophic levels

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The horse-chestnut leaf miner, Cameraria ohridella, is a moth that originates from the Balkans and has invaded most of Europe in about 20 years. In invaded ecosystems, biotic interactions occur at all trophic levels, with diverse consequences. The moth is a serious pest of horse-chestnut, Aesculus hippocastanum, an important ornamental tree in European cities. Although attacked trees do not die, they lose their amenity functions and, as a result, horse-chestnut is gradually replaced by other tree species. The moth also frequently oviposits on sycamore maple, Acer pseudoplatanus, but most larvae die within the first two instars. Attack rates and development success on maple greatly vary from tree to tree. During its invasion, C. ohridella has been adopted by a whole complex of natural enemies, in particular parasitoids, but these play a minor role in the population dynamics of the moth. In Europe, parasitism rates increase with host residence time and proximity to the region of origin, but not with increased biodiversity. Native leaf miners are significantly less abundant in the vicinity of horse-chestnut trees infested by C. ohridella compared to control sites, suggesting apparent competition mediated by shared natural enemies. However, so far, apparent competition through natural enemies has not yet been proven.

Keywords:*Cameraria ohridella, invasive species, biotic interactions, parasitoids, apparent competition*

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S1403TH04

Invasive Species and Quarantine

Plant-mediated interactions between begomoviruses and invasive whiteflies

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Plant-mediated interactions between begomoviruses and whiteflies exert important influences on the invasion of vectors and the epidemiology of plant diseases. Here, we summarize case studies on these types of interactions, and discuss the underlying mechanisms. Present studies show that such interactions can be mutualistic, neutral or negative. For example, infection of tobacco plants with Tomato yellow leaf curl China virus (TYLCCNV) largely increased the performance of two invasive putative whitefly species, Middle East-Asia Minor 1 (MEAM1) and Mediterranean of the Bemisia tabaci complex, but exerted no effect on the indigenous putative species, Asia II 1 and Asia II 3. Likewise, infection of tomato plants by tomato mottle virus had positive effects on MEAM1. In contrast, infection of tomato plants with TYLCCNV had no or only marginal effects on MEAM1 but decreased the performance of Asia II 3. Thus, it seems that positive effects of viruses on the whiteflies are more likely to occur in invasive species than in indigenous species. A recent research demonstrated that when tobacco plants were infected with TYLCCNV, the plant jasmonic acid defenses were repressed but significant changes in amino acid or sugar profiles were not detected, indicating that plant defenses play a major role in mediating the interactions in this system. As plant-mediated interactions between begomoviruses and whiteflies are so complex and variable, more case studies are needed to reveal general patterns of interactions. Furthermore, joined forces of molecular biologists and ecologists are required to reveal the molecular mechanisms in an ecological perspective.

Keywords:*Begomovirus, Bemisia tabaci, invasion, jasmonic acid, plant defense, virus-vector-plant interactions*

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Direct and Indirect Impacts of Infestation of *Myzus persicae* on *Bemisia tabaci*

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The interactions between greenpeach aphid (Myzue persicae), sweetpotato whitefly (Bemisia tabaci) and tomato plants were determined through a combination of four factors: durations of aphid infestation (A), densities of aphids (B), intervals between aphid removal after different durations of infestation and at the time of whitefly release (C), and leaf positions on the plants (D). The results demonstrated that B. tabaci preferred to colonize the plants without aphid infestation when they have a choice. The plants with aphids had fewer whiteflies than the plants that were pre-infested by aphids for a certain period of time and then the aphids were removed. The whiteflies were able to colonize the plants on which aphids once infested, and were able to coexist with aphids if no uninfested plants were available. Direct intensive test revealed that infestation duration of the aphid had stronger defensive effects on whitefly infestation than the density of aphids, and the best combination of induced defense effects on whiteflies were 20 aphids feeding on the plant for 72 h. Indirect orthogonal assays reveal that the prime affected factor of whitefly preferring a host plant among several factors is (C) the interval . The importance of the four factors that affected the induced plant defense against whiteflies can bewas arranged in the following order: (C) > (B) > (A) > (D). In conclusion, the infestation of aphids at a certain density over a certain period of time triggered the plant's defensive system, and induced the plant to defend themselves against whiteflies.

Keywords:*Induced defense, pre-infestation, plant-mediated interaction, orthogonal assays, systemic transport*

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S1403TH06

Invasive Species and Quarantine

Global dynamics of the symbiosis between invasive ambrosia beetles, fungi and bacteria: Insights from high-throughput DNA sequencing

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Ambrosia beetles, the increasingly damaging invasive insects, live in symbiosis with fungi. It has not been tested if the symbiosis also includes bacteria. We combined global sampling, insect anatomy, high-throughput sequencing of bacterial 16S, and multivariate statistics to comprehensively analyze prokaryotic communities in ambrosia beetle mycangium - the organ where beetles carry their symbionts. We tested whether 1) ambrosia beetles also host stable bacterial associates, and 2) whether these bacterial communities are more specific to beetle species or to locality of origin. We analyzed 6 beetle species (Dendroctonus frontalis, Xyleborus affinis, Xyleborus bispinatus-ferrugineus, Xyleborus glabratus, Xylosandrus crassiusculus, and Xylosandrus germanus) that represent 3 types of mycangia, and included five globally distributed species, and four species with major economic importance. Samples of 96 beetle mycangia yielded 1546 bacterial phylotypes. Several bacterial taxa appear to form the core microbiome of the mycangium. Three Mycoplasma (originally thought restricted to vertebrates), two Burkholderiales, and two Pseudomonadales are repeatedly present in mycangia worldwide across beetle species. However, no bacterial phylotypes were universally present, suggesting that ambrosia beetles are not obligately dependent on bacterial symbionts. The composition of bacterial communities is predicted by the host beetle species more than by the locality of origin, which suggests that dominant bacteria are horizontally transmitted rather than acquired from the environment. The invasive Xyleborus glabratus has a unique set of bacteria. We conclude that the mycangium hosts several horizontally transmitted bacteria such as Mycoplasma, most of which are likely facultative commensals or parasites.

Keywords:*ambrosia*

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A trial to evaluate a potential risk of tree-killing diseases by *Raffaelea*-ambrosia beetle complex

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Tree-killing diseases caused by fungi Raffaelea spp. carried by ambrosia beetles are in epidemic in Japan, Korea, and the US. It is likely that invasion of ambrosia beetles in non-indigenous area promote potentially aggressive ambrosia beetle species to create new symbiotic relationship with fungi with high virulence. We determined ecological niche of ambrosia beetles on Qeurcus serrata and evaluated their risks in relation to potential invasiveness and aggressiveness to attack living trees. Freshly cut bolts were set on forest floor in three locations in Japan. Three niche contexts, timing of cutting tree, timing of starting exposure, and wood oldness, were artificially controlled by covering the bolts with metal mesh. Bolts were exposed to insect attacks for one month or until the end of September. The number of entry holes was used as an indicator for abundance of each species. Niche center and niche breadth were estimated for the three niche contexts. Scolytinae tended to show broader niche than Platipodinae. Furthermore, some Scolytid species have a habit of haplodyploidy and sibling mating. On the contrary, all Platypodinae species do not. Therefore, Scolytinae species are likely to have greater potential as alien species. Actually most of the invasive ambrosia beetles in the world belong to Scolytinae with one exception of Megaplatypus mutates. One the other hand, Platypodid species tended to attack fresh bolts compared to Scolytid so that Platypodid have higher risk to vector tree-killing disease than Scolytinae.

Keywords:*ambrosia beetles, invasive species, Japanese oak wilt, Korean oak wilt, Raffaelea spp., laurel wilt*

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Impact of invasive insect herbivores on the foraging behavior of native parasitoids via changes in induced plant volatiles: A comparative study

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Herbivore-induced plant volatiles often play an important role as cues in the foraging behavior of predators and parasitoids in search of prey or hosts. We propose that parasitoids can readily use plant-produced volatiles to distinguish between hosts and non-hosts, but that invading insect herbivores colonizing novel host plants may disrupt these finely-tuned interactions and negatively impact the foraging behavior of native parasitoids. Using Brassica rapa and its complex of native herbivores and parasitoids as a study system, we investigated the effect of two invasive herbivores (Spodoptera littoralis and Heliothis virescens) on the foraging behavior of 3 native parasitoids. In a 6-arm olfactometer setting, we simultaneously tested parasitoid attraction to plants (1) undamaged, (2) damaged by the parasitoid's host herbivore, (3) damaged by a native non-host, and (4) damaged by an exotic non-host. Overall, plants damaged by hosts were in general the most attractive to parasitoids, and undamaged plants the least attractive. Plants damaged by non-hosts showed intermediate levels of attractiveness, but there was no effect of the origin of the non-host (exotic or native) on attractiveness. H. virescens damage was found to cause more parasitoid attraction than S. littoralis damage, showing that invasive exotic herbivores may greatly differ in their impact on native parasitoids. In conclusion, these results demonstrate that invasive herbivores can impact infochemical networks between plants and natural enemies, but that invasive herbivores do not necessarily have a greater impact on specific plant-parasitoid interactions than other native herbivores.

Keywords:*invasive, herbivory, parasitoids, plant volatiles*

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S1403TH09

Invasive Species and Quarantine

Ecology and evolution of invasive plant species and their natural enemies

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Introduced plants become dislocated from their co-evolved interactions with benefical and harmful insects in their native range, and engage in novel interactions in their new range. Biotic theories that try to explain why some introduced plants become invasive in their new range have stressed two benefits of this process for the plant: (1) Plants are released from many of the specialist insect herbivores that exerted top-down control in their native range (enemy release) and (2) When engaging in new interactions in their invaded range, plants benefit from their evolutionary novelty because insect herbivores in the invaded range are naive to their novel secondary metabolites (novel weapons hypothesis), unless they have preadapted to these chemicals on congeneric hosts present in the indigenous community. While some studies support the idea that plants benefit form their evolutionary novelty in the introduced range, there are numerous counter-examples where introduced plants are actually preferred by indigenous herbivores and biotic communities resist invasion of introduced species (biotic resistance). We argue that whether it is the plant or the insect that benefits from the plant's evolutionary novelty will much depend on the predominant mechanism underlying the interaction (e.g. detoxification- vs. recognition-based). Further, rapid evolutionary changes accompany many invasions. Enemy release has been assumed to facilitate an evolutionary shift in investment of resources from defenses against specialists to traits enhancing competition, reproduction, or generalist defense in the novel range (EICA). While support for this theory is mixed, we show some interesting examples compatible with this idea

Keywords:*enemy-release*

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S1403TH10

Invasive Species and Quarantine

Does mutualism drive co-invasion? The case of *Solenopsis invicta* (Hymenoptera: Formicidae) and *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

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Despite mutualism between ants and honeydew-producing hemipterans has been extensively recognized in ecosystems biology, there have been few attempts to test this hypothesis that mutualism between two alien species drive their co-invasion. We try to clarify whether the interactions between S. invicta and P. solenopsis could facilitate mutual population growth and invasive ability. Overall, our results suggest that the mutualism between S. invicta and P. solenopsis facilitate the population increase and fitness of each other. Although native ants may also set up such relationship with P. solenopsis, S. invicta tends to occupy much more honeydew and therefore play a predominant status. Compared with native ants, S. invicta has stronger mutualism with P. solenopsis, which may facilitate their invasion in South China and ultimately achieve co-invasion.

Keywords:*Ant-hemipteran interactions, Solenopsis invicta, Phenacoccus solenopsis, honeydew, co-invasion, colo*

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Will Access and Benefit Sharing procedures impede biological control: The problem

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The objectives of the Convention on Biological Diversity (CBD) are the conservation of biological diversity, the sustainable use of its components (genetic resources) and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. The CBD also recognises the sovereign rights of States over their genetic resources, and the authority to determine access to genetic resources rests with the national governments. In the past, gaining access to biological control agents depended on goodwill, reciprocal arrangements, collegiality, minimal bureaucracy, spirit of sharing of biological control agents and knowledge. In the future biological control may have to comply with the access and benefit-sharing (ABS) regime that is agreed. These issues will be introduced and discussed.

Keywords:*Convention on Biological Diversity, Access and Benefit Sharing, biological control*

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New Access & Benefit Sharing regulations: Is there a future for the biocontrol industry?

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The Convention on the Biodiversity (1992) gives countries the sovereign right over their genetic resources and the right to develop their own regulations for sharing of benefits arising out of utilization of these resources. After the Access & Benefit Sharing Protocol has been agreed in Nagoya in 2010 an increasing number of countries is implementing regulation on ABS, resulting in limited access to new biocontrol agents. This is threatening existing IPM programs as new pests from all over the world are invading agricultural systems and control strategies against these pests have to be compatible with biocontrol strategies against other pests. If a compatible solution is not found in time, chemical intervention will put an end to existing IPM programs. As the biocontrol industry is a small and relatively unknown sector, time and effort to come to agreements on ABS may be unproportionally high and biocontrol companies may decide not to continue investigating a potentially interesting natural enemy. Benefit sharing will increase the cost price of products directly when monetary benefits on the products have to be shared, but also indirectly due to increased administration. Since profit margins in the biocontrol industry are already low, these extra regulations on ABS may put an end to a part of the biocontrol companies. Awareness raising on national decision making level is required to show the impact of strong ABS regulation on biological control and thus on the production of safe food.

Keywords:*Biocontrol Industry, Access and Benefit Sharing Regulations*

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The current status of the conflict between ABS and insect-resource industrialization in Korea

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The recent agreement of ABS (Access to genetic resources and Benefit Sharing) at Nagoya in 2010 requires further action plans by member states to setup PIC (Prior Informed Consent) and MAT (Mutually Agreed Terms) regulations. The Korean government is now preparing the ABS law to be enacted by the end of 2012. The discusssion of how this new regulation will affect the introduction of exotic species including natural enemies of the pest insects as well as the effort for industrialization of insects as biological resources is presented. The legal conflicts among the different governmental institutions in Korea are given an account, and the possible solution is suggested.

Keywords:*ABS, conflict, insect-resource, Korea*

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Access and benefit sharing - a growing challenge for biological control in Europe?

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Exploration for natural enemies, usually in the area of origin of an accidentally introduced pest, and subsequent release in new areas of the pest is the basis for successful biological control independent of whether the biological control agents (BCAs) are applied in inoculative or augmentative control programs. Europe has long been an important exporter of natural enemies for classical biological control. However, over the last decades, Europe has become an increasing importer of BCAs for augmentative use against alien pests. Of the 230 invertebrate species that are commercially available worldwide for augmentative biological control, 170 species are used in Europe. Recent applications of Access and Benefit Sharing (ABS) legislation under the Convention of Biological Diversity have made it impossible or difficult to study, collect and export new natural enemies in certain countries. If the ABS principles are widely applied in this way, biological control will be seriously hampered in Europe. This is particularly the case if the alien pest species originates in a country that applies the ABS rules for BCAs in the expectation of monetary benefit sharing. Whereas it is not expected that the application of ABS procedures will become a significant barrier to the export of BCAs from Europe, European countries are likely to expect documentation that ABS in the source country has been addressed in order to permit importation of BCAs. In the presentation we summarize our concern about the actual and potential adverse effects of ABS rules on the future of biological control in Europe.

Keywords:*alien arthropods, arthropod pests, invertebrate biocontrol agents, classical biological control, augmentative biological control*

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Invasive Species and Quarantine

The effects of Access and Benefit Sharing (ABS) on the utilization of biological control in Brazil

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Brazil has Biological Control Programs comparable to the best one's of the world. In sugarcane, insect pest control is done by using micro or macroorganisms in almost half of the cultivated area. Cotesia flavipes and Trichogramma galloi are released in around 3,500,000 ha to control the sugarcane borer, Diatraea saccharalis, and the fungus Metarhizium anisopliae is sprayed in around 2,000,000 ha to control the froghopper, Mahanarva fimbriolata. However, other Classical or Applied Biological Control programs, using exotic natural enemies, like C. flavipes were also developed in Brazil. There are good results against pests to cassava, soybean, corn, wheat, vegetables, citrus, forestry and pastures. We will discuss the effects of ABS, especially after the Nagoya Convention on the utilization of Biological Control in Brazil.

Keywords:*Biological Control, mass rearing, augmentative biological control*

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Access and Benefit Sharing: Will taxonomic research be facilitated or not?

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In 2010, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits (ABS) was adopted at the tenth meeting of the Conference of the Parties in Nagoya, Japan. For taxonomy, the collection and exchange of specimens in all regions is critical for comprehensive and thorough treatments of biodiversity. Informed ABS policy across countries will be key to ensure that taxonomic research is enabled. Thus, development of recommendations to guide governments when developing ABS policy is paramount to facilitate continued taxonomic research worldwide.

Keywords:*ABS, taxonomic research*

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Challenges for the biological control sector in the implementation of the Nagoya Protocol

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Over the last years, a new international legal architecture on access and benefit-sharing (ABS) for genetic resources has emerged, which have important implications for biological control. The recently adopted Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity constitutes the newest element of this legal framework and adds new impetus to the discussion about how to best address ABS for biological control. The Nagoya Protocol (2010) provides some opportunities to accommodate the special features and needs of biological control in addressing ABS. For example, it requires Parties to consider the importance of genetic resources, including natural enemies, and their special role for food security in the development and implementation of access and benefit-sharing legislation or regulatory requirements. Parties shall also encourage the development and use of sectoral and cross-sectoral model contractual clauses for mutually agreed terms and of voluntary codes of conduct, guidelines, best practices and standards in relation to ABS. Furthermore, the Protocol foresees that in its implementation due regard should be paid to relevant ongoing work or practices under other international instruments and organizations, and opens the possibility for the development of specialized ABS approaches for certain genetic resources or types of uses. This

Keywords:*Biological control, Access and benefit Sharing, genetic resource*

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Climate change and biological control: State of knowledge, risks and opportunities

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A scoping study was undertaken in 2011 for the CGRFA at FAO to ascertain how climate change is likely to affect the use of beneficial invertebrates that are integral to food security and agricultural production. Here we summarise the key findings in regard to the natural enemies that regulate pest populations, with selected case studies. Parasitoids and predators are more likely to be affected by climate change than herbivores because they depend on the capacity of the lower trophic levels to adapt to these changes.In continental regions, most natural enemies will have little difficulty tracking their hosts/prey at predicted range shifts but soil-dwelling and flightless groups may not move as readily and oceanswill prove effective barriers. Therefore, there will be increased demand for biological control agents, especially in island states. Climate change can disrupt the stability of biological control systems through loss of synchrony arising from changes in developmental rates and when species have different initiators of reproductionand diapause. The increasing occurrence of extreme events (heat waves, floods, drought, and unseasonal cold) has the potential to disrupt biological control food webs independently of the disruption of cropping systems. Many classical biological control introductions have been based on few individuals and selection/ introduction of adapted strains to increase genetic diversity may assist system robustness.Genetic diversity of biological control agents can only be satisfactorily maintained in situand the most important reservoirs are agro-ecosystems that favour natural diversity, especially in regions where wild crop progenitors or early domesticated crop lines still persist.

Keywords:*climate change, predator, parasitoid,*

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Assessing the ecological risk posed by a recently established invasive alien predator

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The harlequin ladybird, Harmonia axyridis, is an alien in Europe and many other parts of the world where it affects other species of ladybird through competition for food and intra-guild predation. We developed a method to assess which native ladybird species are most at risk following the invasion of H. axyridis. The three components of the risk assessment are: the likelihood that the assessed native species encounters H. axyridis in the field, evaluated through co-occurrence indices; the effect on native species through competition for food; the effect on native species through IGP. Native ladybirds were scored, for each component, on a three-point scale and the final risk was calculated by multiplying the likelihood of encounter with the sum of the two impact scores. Thirty native European species were assessed through data obtained from field observations, laboratory experiments and literature reviews. Four species obtained the maximum score and are thus considered most at risk: Adalia bipunctata, Adalia decempunctata; Calvia decemguttata and Oenopia conglobata. These species are usually found on deciduous trees, which is the preferred habitat of H. axyridis, have immature stages which are highly vulnerable to intra-guild predation by H. axyridis and are primarily aphidophagous. This risk assessment method could be applied to other invasive alien predators which are considered a threat to other native species through competition and predation.

Keywords:*Harmonia axyridis, European ladybirds, Ecological Impact, Risk assessment, Predators*

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Does knowledge of natural host range always help predict host range in new areas of introduction? A case study with the braconid parasitoid *Microctonus aethiopoides* Loan

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Information on natural and novel host range of proposed biological control agents can help to inform predictions of potential host range in new areas of introduction. A retrospective study was conducted to compare the natural host range of Microctonus aethiopoides Loan (Hymenoptera: Braconidae) with its novel host range found in Australia and New Zealand, where it has been introduced to control the adult stage of the weevil Sitona discoideus Gyllenhal (Coleoptera: Curculionidae), a pest of lucerne. The host ranges determined from field surveys in Australia and Morocco were compared with field host range in New Zealand. In Morocco, species in the genera Sitona and Charagmus (Curculionidae: Entiminae: Sitonini) and Hypera (Curculionidae: Hyperinae: Hyperini) were found to be parasitised by M. aethiopoides. In Australia, non-target parasitism of 'Prosayleus' sp. 2 (Curculionidae: Entiminae: Leptopiini) was the only non-target host found for M. aethiopoides. In New Zealand 19 native and introduced weevil species have been recorded as hosts of M. aethiopoides, mainly in the subfamily Entiminae (tribe Leptopiini) but also in Curculioninae, Cyclominae and Lixinae. This is discussed in the context of predictions that could have been made at the time of introduction 30 years ago had the Moroccan and Australian data, modern molecular technologies and current understanding of weevil classification been available. The absence of Leptopiini in Morocco and the record of a native Australian leptopiine host could have indicated that native weevils in this tribe in New Zealand might be at risk of attack by M. aethiopoides.

Keywords:*Microctonus aethiopoides Loan, Sitona discoideus Gyllenhal, non-target parasitism, natural host range, novel host range, Morocco, Australia, New Zealand*

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Assessing risks of post-introduction shifts in host specificity of parasitoids introduced to control aphids

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Assessing the risks of post-introduction shifts in host specificity of parasitoids introduced for biological control is crucial for the safety of introductions. Assessments are based on reviews of the literature on host specificity and candidate biology, field evaluations of host specificity in the region of origin, and quarantine experiments. Each of these approaches has its strengths and weaknesses. Using assessments of risks of host shifts in Aphelinus species proposed for introduction against aphids, I discuss ways to improve assessment of the risks of host shifts, with an emphasis on quarantine experiments. Host specificity shifts may be behavioral or genetic, and experiments have allowed us to assess the risks of both types of shift. Surprisingly, host specificity does not appear to be behaviorally dynamic, at least in Aphelinus species with narrow host ranges. On the other hand, laboratory selection gave a more rapid shift in host specificity than we had expected. Analysis of the genetic architecture should provide better understanding of the risks of evolutionary host shifts.

Keywords:*parasitoids, aphids, host specificity, behavior, genetics, selection, evolution*

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Biological Control and the carbon cycle

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Biological control can lead to decreases in the use of pesticides and therefore reduced carbon emissions associated with pesticide manufacture, transport and application. I present an analysis of the extent to which naturally-occurring biological control of the soybean aphid, Aphis glycines, leads to reductions in carbon emissions in the United States. I also report calculations for carbon emission reductions associated with selected classical biological control projects. Finally, I discuss the potential role of classical biological in the carbon cycle more generally, including effects on carbon sequestration.

Keywords:*carbon footprint, biological control, Aphis glycines*

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Brown marmorated stink bug, *Halyomorpha halys* (Stal): Impact of an Asian species introduced into North America

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The brown marmorated stink bug (BMSB), Halyomorpha halys (Stal) is an insect native to China, Taiwan, Korea, and Japan accidently introduced into the United States sometime in the mid- to late 1990s that has emerged as a pest of unprecedented importance to agriculture. Currently, BMSB is well established throughout DE, MD, PA, NJ, VA and WV and has been officially detected in over 35 states in the U.S. and recently in Ontario, Canada. BMSB is considered a polyphagous pest of many specialty crops in Asia including tree fruit, vegetables, shade trees and leguminous crops with specific mention of apple, cherry, peach, and pear. After detection, localized reports of problems were reported in and near Allentown, PA in the early 2000s. However, in 2008, BMSB populations increased dramatically and by 2010 attacked many specialty and row crops in the mid-Atlantic region of the United States. In apple alone, BMSB is estimated to have caused $37 million dollars in damage. In addition, this insect is a serious nuisance pest, as overwintering populations will move to human-made structures. In order to combat this serious pest, a large team comprised of researchers from 14 institutions in 10 states in the U.S. was rapidly assembled and is currently developing monitoring tools and management strategies for a variety of cropping systems.

Keywords:*brown marmorated stink bug, pest, invasive,*

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Observations on current and future *Drosophila suzukii* distribution, impact and management in the United States

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Drosophila suzukii, Spotted Wing Drosophila, is a pest of fresh commercial stone and small fruit in the United States of America. It was recorded in commercial crops in Pacific production areas during 2009. Subsequent recordings of D. suzukii and fruit damage associated with this pest were made in all major Pacific, Midwest and Atlantic fruit production areas. The distribution and impact of D. suzukii on various fruit industries in each of these regions will be discussed. Key differences of D. suzukii seasonal phenology and damage on selected crops will be described for each of these production regions. The currently used methods to manage D. suzukii, and how management between areas may differ will be discussed. Promising future management strategies of this pest will be elucidated.

Keywords:*Drosophila suzukii, Spotted Wing Drosophila, United States distribution*

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Invasive Species and Quarantine

Urban and agricultural impact of *Megacopta cribraria* in the southeastern United States

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In October 2009, Megacopta cribraria (Heteroptera: Plataspidae) was discovered aggregating on south and east facing exterior surfaces of buildings in Georgia, United States. This invasive insect was previously unknown to the Western hemisphere. The insect is native to Asia where its preferred host is kudzu (Pueraria spp.). In a little more than two years, the distribution has spread to nearly all of Georgia and South Carolina, and parts of Alabama, North Carolina, and Virginia. Genetic analyses from the entire North American population suggest a single maternal lineage. Starting in 2010, large populations were observed on soybean, Glycine max, where an entire generation was completed. Populations in soybean can exceed 100 individuals per sweep and inflicted an average yield loss of 37% compared to adjacent plots that were treated with insecticides to prevent M. cribraria colonization. Studies in Georgia show that there are at least two generations and three distinct periods of adult flight activity per year. During the spring and fall, M. cribraria is a serious urban nuisance as it flies to and lands on light colored surfaces including people, homes, and automobiles. The pest gives off a strong odor when disturbed and crushing on human skin will result in localized itching and skin whelps. Continued range expansion is expected.

Keywords:*invasive insect*

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Invasive Species and Quarantine

Asian citrus psyllid and Huanglongbing in the New World: Impact and anagement

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Citrus evolved in Asia, and so too have many of its diseases, pests and their natural enemies. These include huanglongbing (HLB), its psyllid vector Diaphorina citri, and the parasitoid Tamarixia radiata. Little attention was paid in 1942 to the first record of D. citri in Brazil, but the discovery there of huanglongbing 62 years later, followed by Florida in 2005, marked the beginning of radical changes to the citrus industry. Intensified insecticidal control and plant nutrition, coupled with protected nursery production and roguing of infected trees, have been moderately effective in managing the disease but greatly increased production costs. Vector control is key, even in infected orchards, and has proven more efficient in large production units, leading toward consolidation and/or coordinated area wide sprays. However, problems of secondary pest resurgence signal the need for an integrated approach employing cultural methods and selective, even threshold driven applications that conserve natural enemies and may open the door for augmentative biological control. Progress toward these goals include: sprays directed at adult psyllids during plant dormancy that spare many beneficial insects, an efficient sampling method, the "stem tap" to quickly assess psyllid populations, organization of mass rearing facilities for T. radiata, and a growing number of available selective insecticides. With these tools in hand, the industry can survive while awaiting a genetic "magic bullet" to wipe out HLB.

Keywords:*Asian Citrus*

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Invasive Species and Quarantine

Monochamus species as potential vectors of the pine wood nematode in Europe : What can be learnt from the Asian situation ?

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The worldwide genus of long-horned beetles, Monochamus, has drawn a particular attention because several species are known to be a vector of the Pine Wood Nematode (PWN), Bursaphelenchus xylophilus, carrying and transferring the nematode to conifer host trees. Although not a pest in its native North American range, PWN has devastated forests in Asia, and more recently in Europe. The five Monochamus species recorded in Europe, i.e. M. galloprovincialis, M. sutor, M. sartor, M. urussovi and M. saltuarius, may represent potential vectors for PWN. However, only M. galloprovincialis has been proved so far to be associated with PWN invasion of Portugal and Spain. This talk will review the European Monochamus species, with a special focus on their geographic distribution range and ecological requirements. A state-of-the-art of the present situation of pine wilt disease in Europe will be presented, and the potential vectoring role of the different Monochamus species throughout Europe will be evaluated with regard to the situation in Asia. Finally, we will present the ongoing European project REPHRAME that is bringing together Europe's leading experts on PWN, together with colleagues from around the world, to address the key gaps in current knowledge of pine wilt disease and its associated organisms.

Keywords:*Monochamus species, Pine Wood Nematode, Europe, REPHRAME*

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Asian soybean aphid in North America ? Invasion and classical biological control

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The soybean aphid, Aphis glycines, is native to eastern Asia and was first found in North America in the year 2000. We discuss molecular data regarding the Asian provenance of soybean aphid in N. America and also the possibility of more than a single invasion. Soybean aphid requires shrubs in the genus Rhamnus to overwinter, and its invasion was facilitated by the previous invasion of Rhamnus species from Europe and Asia. Also, the presence of soybean aphid in North America is likely facilitating the spread of non-native aphid consumers such as the multicolored Asian ladybeetle, Harmonia axyridis. Together, the network of interactions among introduced species linked to soybean aphid may be considered an 'invasional meltdown'. Classical biological control of soybean aphid using Asian parasitoids is underway and we discuss these intentional introductions in light of the unintentional introduction of a parasitoid ? Aphelinus certus ? that is attacking soybean aphid.

Keywords:*Aphis glycines, Aphelinus certus, Binodoxys communis, biological control, Rhamnus, Harmonia axyridis, invasional meltdown*

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The citrus flatids, *Metcalfa pruinosa*, recent outbeak in the Korean Peninsula

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The citrus flatid planthopper, Metcalfa pruinosa (Say 1830) (Hemiptera: Flatidae), has a native distribution in eastern North America, It has recently invaded Italy in 1979 and has since spread to other European countries. In 2009, Metcalfa pruinosa was discovered in Seoul and the Gyeonggi Province, Republic of Korea. This is the first record in the eastern part of Palaearctic. One year after its discovery, in July 2010, we found significant populations and serious damage on many deciduous forest trees, ornamental trees, and agricultural crops in central regions of the Korean Peninsula. In this paper, we report the status of the outbreak and discuss the biology, morphological characters, distribution, host plants, and the importance of M. pruinosa as a potential insect pest in the Korean Peninsula.

Keywords:*Metcalfa pruinosa, outbreak, Korea*

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Invasion of leafminers, *Liriomyza* spp. (Diptera: Agromyzidae), in the eastern Palearctic region

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Liriomyza sativae Blanchard, L. trifolii (Burgess) and L. huidobrensis (Blanchard) (Diptera: Agromyzidae) are indigenous to the New World, but they have established populations in the eastern Palearctic region in the last two decades. These three species are polyphagous herbivores that are important pests of numerous vegetable and ornamental crops. Two of the most important of them, L. sativae and L. trifolii, have been introduced into the same geographic areas and species displacement between these two species has been observed. In Hainan Island, China, L. trifolii replaced L. sativae as the predominant leafminer of vegetables, as in the western USA in the 1970s. However, in Japan, L. trifolii was displaced by L. sativae. In Hainan Island, where the management of Liriomyza depends mainly on chemical insecticides, the rapid displacement of L. sativae by L. trifolii can be attributed to the lower insecticide susceptibility of L. trifolii. In contrast, the displacement in the opposite direction in Japan is probably due to the higher fecundity of L. sativae and differential effects of the introduced natural enemy Dacnusa sibirica Telenga (Hymenoptera: Braconidae). In winter, L. sativae and L. trifolii are found only in greenhouses in Japan, and D. sibirica is a more effective parasitoid at low temperatures. When growers in Japan find the mines induced by Liriomyza on vegetables in greenhouses in winter, they usually use D. sibirica to control the leafminers. This parasitoid can control L. trifolii effectively, but cannot control L. sativae.

Keywords:*species displacement, insect pest, Dacnusa sibirica, natural enemy, parasitoid, fecundity*

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Invasion of exotic gall midges in East Asia

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Recentry, the gall midges, which invaded in to Korea, severely damaged on plants in agriculture and forest. Five invasive gall midges are economically important, and the information on their biological features, invasive pathways and economic impact is provided. Pine gall midge introduced from Japan. It have spreaded from Mokpo in southwestern port, distributed nationwide in present. It damaged on Korean red pine and black pine, prevention cost was needed each year. It is very important pest in Korean pine tree. The black locust gall midge was discovered in 2001 for the first time in Korea and have spread into all Korean peninsular and Jeju island. According to Dr. Yukawa, its origin is USA in North America, and invaded into countries where USA troop stationed, as like Japan and Korea in Asia, and Balkan peninsula in Europe. The orchid gall midge was occurred in greenhouse for Dendrobium spp. occasionally. It attacked on flower buds and they were rotted and dropped into ground. It was introduced through young seedlings from Thailand. After control by insecticide, the orchid gall midge were eradicated in greenhouse in which the orchids were damaged. Last, the blueberry gall midge was newly invaded into Korea. It is major pest for Vaccinium spp, its origin is eastern USA. Its pathway was suspected because of many seedlings. The monitoring and fast control is more important at post quarantine, to prepare increase of trade of agricultural commodity after FTA with other countries.

Keywords:*gall midge, Eastern Asia. quarantine,*

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Dispersal patterns of exotic forest pests in South Korea

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Invasive species have potentially devastating effects on ecological communities and ecosystems. To understand the invasion process of exotic forest pests in South Korea, we reviewed four major species of exotic forest pests: the pine needle gall midge (Thecodiplosis japonensis), pine wilt disease caused by the pine wood nematode (Bursaphelenchus xylophilus), the fall webworm (Hyphantria cunea), and the black pine bast scale (Matsucoccus thunbergianae). We consider their biology, ecology, invasion history, dispersal patterns and related traits, and management as exotic species. Among these species, the dispersal process of fall webworm was linear, showing a constant range expansion as a function of time, whereas the other three species showed biphasic patterns, rapidly increasing dispersal speed after slow dispersal at the early invasion stage. Moreover, human activities accelerated their expansion, suggesting that prevention of the artificial movement of damaged trees would be useful to slow expansion of exotic species. We believe that this information would be useful to establish management strategies for invasion species.

Keywords:*Forest pest*

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The status quo of invasive alien insects species and plant quarantine in Korea

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The recent increasing agricultural commodities from abroad and travelers with the economic growth in Korea and globalization have resulted in an exponential increase in the number of invasive alien insect species establishing in Korea. When the 'alien' is defined as the species introduced after the beginning of the Greater Korean Empire Era (1897), a total of 171 insect species is proposed as invasive alien species. From this study, on average, 0.85 alien insect species has become established each year for the last 40 years, and 76.6% of the alien insect species were economic agricultural pests. The annual invasion rate and the pest interceptions from imported cut flowers, planting materials, and vegetables have been rapidly increasing. Passengers' baggage is an important pathway for fruit flies. Most of the alien pests that were first found in Japan were confirmed in Korea at least 3 years later until the 1990s, but the pattern has been often reversed since the 2000s. So the continuous information sharing with neighboring countries like Japan and China is needed to develop the system for early detection and prompt action against invaders.

Keywords:*invasive alien insect species, plant quarantine, Korea*

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Biological control options of the invasive tomato pest *Tuta absoluta*

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The South American tomato pinworm, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating pests in tomato crops. In the absence of control strategies, damages in tomatoes (Lycopersicon esculentum) can reach 100%. Although originally from South America, T. absoluta was detected in eastern Spain at the end of 2006, and in the following years in main Mediterranean coastal tomato-producing areas (and more generally Europe) with populations quickly reaching damaging levels. This occurred despite plant protection agencies' efforts, demonstrating how fast T. absoluta populations can build up when resources are available. In newly infested areas, T. absoluta is especially problematic during the first years of its presence. Nevertheless, after two to three years in certain areas, the incidence of T. absoluta has become less severe. There are several factors contributing that explain this reduction in T. absoluta's incidence, such as an increase in farmers' and producers' knowledge of pest behavior and biology and the correct application of integrated pest control strategies. The impact of opportunistic native natural enemies (fortuitous biological control) should be considered as one of the key factors in this reduction. Furthermore, successful biological control programs based in the use of predatory mirids (Hemiptera: Miridae) have contributed not only to manage T. absoluta but also other important pests of tomato such as whiteflies. This strategy has been successfully implemented in more than 6000 ha of commercial greenhouses in the Mediterranean basin.

Keywords:*tomato, Nesidiocoris tenuis, Macrolophus pygmaeus, Necremnus artynes, Trichogramma achaeae*

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Introduction of natural enemies against exotic insect pests in Japan

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Exotic natural enemies have been introduced for controlling exotic pests after the world war in Japan. Some species have been introduced for the classical biological control of introduced pests of fruit trees. Successful examples are introduction of two aphelinid parasitoids, Aphytis yanonesis and Coccobius fulvus against the arrowhead scale, Unaspis yanonensis, a scale insect attacking citrus trees, and that of a torymid parasitoid, Torymus sinesis against the chestnut gall wasp, Dryocosmus kuriphilus. Protected culture has been developed dramatically in Japan in 1960s. Many exotic species or strains of arthropod pests infesting greenhouse vegetables have invaded with the increase of international trade of seedlings. Most of them are small in size but highly reproductive and easily develop resistance to pesticides. The most serious groups are whiteflies, e.g. Bemisia tabaci type B and Q, Trialeurodes vaporariorum, and thrips, e.g. Thrips palmi, and Frankliniella occidentalis. Aphelinid parasitoids, Encarsia formosa, Eretmocerus eremicus and Eretmocerus mundus and a predatory bug, Amblyseius swirskii have been introduced to control whiteflies. A predatory mite, Neoseiulus cucumeris has been introduced to control thrips. Commercial augmentative release of these species is carried out in greenhouses. They have been commercialized in Europe and imported by trading companies. These exotic whiteflies and thrips transmit virus diseases of some greenhouse crops, which hamper biological control of these pests using natural enemies. Present major concerns about importation of exotic natural enemies are their environmental impact and the procedure of access and benefit sharing.

Keywords:*introduction, natural enemy, Japan, exotic pest*

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Climate change and pest risk analysis

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Projected climates are predicted to influence the growth, development and behaviour of pest and pathogens necessitating the revision of current biosecurity practices such as preparedness, prevention, and containment policies and trade / market access issues. A review led by the Quadrilateral Biosecurity Climate Change Working Group considered the implications of climate change on biosecurity policy and recommended that a template be developed for incorporating climate change projections into Pest Risk Analysis (PRA) to enhance biosecurity decisions. PRA's involve pest identification; pest risk assessment (categorisation, assessment of probability of entry, establishment and spread, assessment of consequences) and pest risk management. We discuss the key elements required to incorporate the consideration of climate change into PRA's with the view for developing a prototype template for use in biosecurity policy arenas. Also discussed are procedural guidelines for possible inclusion such as the use of a standard climate change emission scenario, nominating a specified time horizon (eg 2020-2030 as opposed to longer range forecasts) and consideration of the climate change parameters to be included. Projections of future climate trends indicate that the nature, extent and intensity of climatic change will vary spatially and temporally. Responses of pests and pathogen to these changes are expected to be species and region-specific and made more complex by their interactions and interdependencies. Determining the effect of climate change on pest risk analyses will lead to new directions in biosecurity management and implications for biosecurity policy benefiting by directing scarce resources where they are most required.

Keywords:*climate change, pest risk analysis, pest risk assessment*

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S1405TH15

Invasive Species and Quarantine

Lessons from the global eradication and response database

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A database of insect and plant pathogen eradication programs has been developed currently, with 916 incursion responses, including 855 eradication programs from 103 countries, documented with 580 references. Of these, 143 arthropods were targeted in 669 eradication programs in 91 countries, with a surprisingly high rate of 280 eradications (intentional local extinctions) confirmed, 98 known failures, 8 false declarations of success and 85 programs in progress (86 of unknown outcome). The most frequent targets were gypsy moth (73), medfly (57), Oriental fruit fly (40), yellow fever mosquito (33) and emerald ash borer (25). Many lessons can be learned from a historical analysis of the targets and technologies used, including surveillance systems and eradication tools. This paper will present a range of analyses from the database that support the proposition that insect eradication is often possible and cost effective for organisms where the alternative is significant long-term ongoing costs.

Keywords:*Global Eradication*

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Acarology

A immunomodulatory protein RH-p36 is relating to hard tick blood-feeding success and oviposition

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A immunomodulatory protein RH-p36 was identified in the tick Rhipicephalus haemaphysaloides. The cDNA sequence of RH-p36 is 844 bp, and it encodes a deduced 218 amino acid protein with a size of 24 kDa. Bioinformatic analysis shows that RH-p36 is high homology to the immunomodulatory protein p36 of tick Dermacentor andersoni. The recombinant protein expressed in insect cell sf9 suppressed T-lymphocyte mitogen-driven in vitro proliferation of splenocytes as well as the expression levels of several cytokines such as IL-2, IL-12 and TNF-α. Furthermore, the proliferative response of splenocytes isolated from rRH-p36-inoculated mice was significantly lower than for those from control mice, suggesting that rRH-p36 could also directly suppress immune responses in vivo. Interestingly, microarray analysis of the splenocytes showed that the expression of several immunomodulating genes was down-regulated by rRH-p36 inoculation. Disruption of the RH-p36 gene with RNAi led to a 42.5% decrease in the tick attachment rate 24 hours after introduction in the rabbit ears and a 47.5% decrease in the engorgement rate of ticks. Unexpectedly, the RNAi also led to significant effects on tick oviposition. These effects of RH-p36 on blood feeding and ovipisition were further confirmed by vaccine tests using the recombinant protein. These results indicate that RH-p36 is a novel member of immunosuppressant protein involved in tick blood feeding and ovipisition.

Keywords:*tick, immunosuppressant protein, RNAi, feeding and oviposition*

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Characterization and movement of iron binding proteins on *Haemaphysalis longicornis*

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Ticks are obligate hematophagous parasites, utilizing blood from their hosts for survival and reproduction. Ticks require large amount of blood, exposing them to great amount of free iron. Iron is both essential and toxic for living organisms. Hemoglobin and ferric-transferrin in vertebrate host blood are the two major iron sources for ticks. Different organisms have several mechanisms for iron metabolism. Among the many aspects of iron biology, two abundant proteins involved in iron transport and storage, ferritin and transferrin (TF). Ferritins are found in prokaryotes, plants and animals, and are characterized by high capacity for iron storage; TF is found in multicellular animals and is characterized with high affinity for iron binding. In ticks, however, iron metabolism remains to be unclear. Here, we analyzed that movement of intracellular ferritin (FER1), secreted ferritin (FER2), and TF obtained from host blood in the hard tick, Haemaphysalis longicornis. Through western blot and indirect immunofluorescent analysis using specific anti-ferritin mouse sera, a similar pattern of expression in different organs and developmental stages was observed for both ferritins. However, only FER2 was detected in the egg and hemolymph. Although intact TF was detected thorough depvelopmental stages, TF was detected in the midguts, hemolymph, and ovaries at tick tissues of adult stage after engorgement. These results indicate that these proteins play a critical role in meal iron transport and iron storage in eggs in Haemaphysalis longicornis.

Keywords:*Ferritin, Transferrin, Iron*

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The role of autophagy in the hard tick *Haemaphysalis longicornis*

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The life cycle of ticks is characterized by alternate off-host and on-host conditions. The generation time is estimated at several years and most ticks spend more than 95% of their life off the host. To understand how ticks obtain energy over a long period of nonfeeding (starvation), we focused on autophagy, a crucial proteolysis system via the lysosomes for various cellular processes that is induced during starvation in eukaryotes. To date, we have isolated 5 homologues of autophagy-related (ATG) genes (ATG3, ATG4, ATG6, ATG8 and ATG12) from the 3-host tick Haemaphysalis longicornis. We have also found autophagic organelles in digestive cells of the midgut of unfed female ticks. In the present study, to determine the relative transcription levels of HlATG genes, real-time PCR was performed using specific primers. The expression levels of each HlATG gene were higher at the egg stage compared with all active stages (larval, nymphal, and adult stages). At nymphal to adult stages, the expressions of HlATG genes were elevated at the premolting stage (during metamorphosis). These results suggest that HlATG genes may function at nonfeeding periods such as the egg stage and metamorphosis. RNA interference (RNAi)-mediated gene silencing of HlATGs are in progress to analyze the function of HlATG genes at nonfeeding periods of the tick. On the basis of these results, the role of autophagy in the tick will be discussed.

Keywords:*tick, Haemaphysalis longicornis, nonfeeding period, autophagy-related genes, autophagy*

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Genes and models to understand dispersal: What do they tell us about the current and potential invasive route of the tomato red mite, *Tetranychus evansi*?

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Dispersal is an important parameter to help understanding species evolution and adaptation. In the case of crop pest, knowing current dispersion pathways and also predict its potential geographical distribution, is particularly useful for species with strong invasion abilities. However dispersion often escapes direct observation in natural populations of small organisms. Population genetics theory predicts that variations of neutral allele frequencies in space can provide a reliable indirect measure of the amount of genetic exchange, and thus of migration of individuals inside a species. Based on examples in spider mites, I will review several features (residence status, natural vs. agricultural, host range, etc) having an influence on population genetic structure. I will then focus on an invasive spider mite, Tetranychus evansi. Native of South America, the mite has emerged as a new destructive pest of solanaceous crops worldwide. Using recent developments in Approximate Bayesian Computation (ABC) and variation in multi-locus genetic markers, the complex historical demography of this cryptic invasive pest could be reconstructed. By distinguishing among multiple pathways and timing of introductions, ABC methods provide insights into complex invasion scenarios and understand pass dispersion pathways. In addition, model distributions informs on the future potential range of the invasive mite, one of the key steps to prevent further spread.

Keywords:*Population genetics, invasions, spider mites, gentic markers, ABC methods, microsatellites*

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Worldwide journey of invasive alien mites and ticks

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Japan is a great economic and depending greatly on importation of natural resources from abroad. So, Japan has always a high risk of biological invasion. Of course, Japan has quarantine systems against alien species adverse to ecosystems, agricultures, forestry, fisheries and human health. However, the invasion risks of alien species continue rising rapidly accompanied by economy globalization. Mite invasion also cannot be an exception. Alien species adverse to natural ecosystems are regulated by the Alien Species Act in Japan. But the law focuses only on visibly recognizable species, so that species too small to see such as virus, bacteria, fungus and mites are beyond of the scope of this law. Alien pests harmful to agricultural crops, including mites, have been severely restricted to be introduced into Japan by the Plant Protection. But recently, the pressure of global trade liberalization has made the law to release of regulations of various pests including spider mites. Infectious diseases and their career species are quarantined by Rabies Prevention Law, Domestic Animal Infectious Diseases Control Law and Human Infectious Diseases Control Law. But these laws cannot cover the wild life diseases. Most serious problem is that wild reptiles which can be careers of ticks and tick borne diseases can be introduced freely. These loopholes of Japan's regulation systems have actually caused mite and tick invasions, which will result in serious problems for mite diversity as well as human society.

Keywords:*Biological invasion, Alien species, plant protection, spider mite, tick born disease, WTO*

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Population structure of phytoseiid mite *Neoseiulus womersleyi* and its perspective on conservation biological control

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In conservation biological control reducing pesticide sprays, preservation of natural enemies by habitat management is thought to enhance their activity. However, it is essential to elucidate the population genetic structure of natural enemies to better conserve them. Here, some topics on the predatory mite Neoseiulus womersleyi (Acari: Phytoseiidae) will be introduced and discussed. N. womersleyi is an important predator of the Kanzawa spider mite, Tetranychus kanzawaki, in tea fields. To conserve them by banker plant systems, population structure of N. womersleyi in a tea field was analyzed by using microsatellite DNA markers. The kinship coefficients between individuals did not differ significantly within a site, but the coefficients gradually decreased with increasing distance. Therefore, the management unit for conservation of N. womersleyi should be this approximate scale. In commercially grown chrysanthemums are well protected from lepidopteran pests by net barriers; however, some small insects and mites have developed resistance against chemical sprays. Here, we used DNA markers to identify the species of phytoseiid mites around the chrysanthemum fields. The dominant phytoseiid mite was N. womersleyi, which accounted for more than 70% of those trapped and identified. Next, their population structure was analyzed using microsatellite markers. Spatial autocorrelation indices were low at all distance ranges, indicating that a high level of gene flow occurred across this area. It is possible that frequent mowing of the area around the fields might enhance dispersal of the phytoseiid mites, and thereby account for the observed population structure of the species.

Keywords:*phytoseiid mite, conservation biological control, population structure, microsatellite DNA marker*

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Patch-leaving decision of the predatory mite based on multiple signals

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How to find a prey patch and how long to stay there are two major factors determining the foraging efficiency of predators and parasitoids. Many predators and parasitoids use herbivore-induced plant volatiles as cues for their foraging behaviors in many systems consisting of plants, herbivores, and natural enemies. For example, the predatory mite Neoseiulus womersleyi, which is a specialist predator of tetranychid mites, is attracted to volatiles emitted from plants infested with their prey. Once predators have located a prey patch, multiple pieces of information are available to decide their residence time in the patch. Contact chemicals associated with prey and competitors, and volatiles from plants would exhibit the condition of the currently inhabiting patch. The volatile chemicals from outside the patch are important information to evaluate the surrounding area. The resource level in habitat can be estimated by foraging experience of predators. Our experiments using small leaf disks showed that the residence time of N. womersleyi was short when prey-related products such as exuviae, feces and webbing were on a prey patch. The residence times of predatory mites was affected by the presence of HIPV from outside the patch and previous foraging experiences. N. womersleyi appears to determine residence time in the current patch based on the resource level in the current patch and that in the surrounding habitat.

Keywords:*residence time, herbivore-induced plant volatiles, Neoseiulus womersleyi, multiple information*

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Acarology

Local movement of prey-predatory mites and implementation in management

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Two spotted spider mite and European red mite are the important pests in apple and pear in Korea. In fruit tree system, twospotted spider mite moves more frequently and widely than European red mite. Neoseiulus womersleyi is the dominant among spider mite predator guild. We have studied the movement pattern of Tetranychus urticae and N. womersleyi within tree, between tree, within orchard, and even among orchards and surrounding. Overwintered adult T. urticae moved from the tree trunk to the ground vegetation mostly from late March to mid April with a daily peak activity from noon to 4 p.m. Movement of overwintered N. womersleyi from the tree trunk to ground was negligible, mostly because of the different overwintering habitat. Movement of T. urticae from the ground vegetation to apple tree began from mid May and that of N. womersleyi followed in two weeks. When the densities of T. urticae and N. womersleyi were high in the tree canopy, dispersal movements of both species occurred by downward ambulation or aerial take-off. In aerial movement, distance of the dispersal ranged less than 100 m with exponential decay pattern. However, significantly longer transportation events were also noticed, but not experimentally validated. Summarizing, we present options for better mite management tactics.

Keywords:*Neoseiulus womersleyi, Tetranychus urticae, specialist, generalist, dispersal*

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Belowground fungus adaptively modulates the foraging behavior of an aboveground plant-inhabiting predator

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Arbuscular mycorrhiza, the association between mycorrhizal fungi and plant roots, is a ubiquitous phenomenon but whether and how mycorrhiza affects aboveground herbivore-carnivore interactions is largely unknown. On bean plants Phaseolus vulgaris, the mycorrhizal fungus Glomus mosseae enhances the fitness of the herbivorous two-spotted spider mite Tetranychus urticae and its natural enemy, the predatory mite Phytoseiulus persimilis, via changes in host plant and prey quality, respectively. Thereupon, we hypothesized that foraging P. persimilis are able to recognize mycorrhiza-enhanced prey quality directly via prey-related cues and indirectly via herbivore-induced plant volatiles (HIPV). We assessed the response of foraging P. persimilis to direct and indirect cues using choice cages and a Y-tube olfactometer, respectively. P. persimilis used direct prey-related cues, mainly derived from eggs, to discern prey quality and preferentially resided and oviposited close to prey fed on mycorrhizal plants. P. persimilis responded strongly to HIPVs emitted by plants infested for six days and preferred HIPVs of mycorrhizal plants to those of non-mycorrhizal plants. In contrast, the predators were little responsive and indiscriminative regarding mycorrhization when exposed to volatiles emitted by non-infested plants. Analysis of the HIPV composition by proton-transfer-reaction mass spectrometry (PTR-MS) revealed that mycorrhiza increased the emission of β-ocimene and β-caryophyllene, two compounds synthesized de novo upon spider mite attack. Our study shows that plant-inhabiting carnivores may recognize mycorrhiza-induced changes in the quality of herbivores as prey via both direct and indirect cues, and provides a key example of an adaptive indirect interaction between a belowground microorganism and an aboveground predator.

Keywords:*Acari, predatory mites, coevolution, foraging, predator-prey interaction, mycorrhiza*

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Aerial dispersal of *Aceria carvi* (Acari: Eriophyidae)

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Aceria carvi Nal. is a serious pest of biennial caraway (Carum carvi L.) in Europe. The mite attacks plant tissues and causes development of flower galls which produce no seeds thus reducing yield substantially. It was presumed that the pest relies on dispersal by wind currents for transfer from the field of senescent plants to the field of young plants during summer. Airborne dispersal of A. carvi was recorded by trapping mites in petroleum jelly on microscope slides mounted vertically on wood stick 1.5 m above ground level. The effect of wind speed on mite behavior was studied under laboratory conditions using a specially made wind tunnel allowing microscopic observation. The results revealed that number of mites caught on sticky traps increased in time till about four weeks before the caraway harvest. A positive correlation between A. carvi density on caraway umbels and number of airborne mites was found. Wind-tunnel experiments showed that adult females actively entered the air current at speed > 0.5 m/s by orienting their bodies and using the caudal lobe as a springing mechanism. The greater the wind speed, the sooner the mites were carried aloft.

Keywords:*caraway, gall mites, dispersal behavior, sticky traps, wind tunnel*

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Epidemiology of ticks (Acari: Ixodidae) infesting buffaloes (*Bos Bubalus bubalis*) in district Toba Tek Singh, Punjab, Pakistan

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The current research was conducted in order to study the epidemiological parameters on the prevalence of tick infestation and associated risk factors in buffaloes of district Toba Tek Singh of central Punjab, Pakistan. The prevalence of ticks in buffaloes was recorded as 31.21% (352/1128). Among various species of ticks, the prevalence of Hyalomma marginatum (20.12%) was higher ($P<0.05$; OR= 1.75) as compared with Rhipicephalus microplus (11.08%). Various catagories of age, sex and breed of the host were found equally susceptible ($P>0.05$) to ticks. In different observed management and husbandry practices, the prevalence of ticks was found higher in animals kept on un-cemented floor (15.51%; OR= 2.17) followed in order by partially cemented (9.39%; OR=1.29) and cemented floor (6.29%; 71/1128) while feeding system, housing system and animal keeping were not found associated with the prevalence of ticks ($P>0.05$). Variation in infestation of ticks at different body parts including the head, neck, ear, dewlap, back, abdomen, foreleg, shoulder, hind leg, congenital areas and the tail was recorded which were ranges from highest at inside thigh (17%) to lowest at rump. In the present survey, highest prevalence was recorded in July and lowest in December. Comparison of hematological changes shows remarkable differences between infested and non-infested animals, in the form of low values of infested animals whereas an increment in biochemical parameters values has been observed in tick infested animals. The results of the present study may implicit significant data for the for better planning tick control program in the study area.

Keywords:*Prevalence, Epidemiology, Ticks, Bos bubalus bubalis, Toba Tek Singh, Punjab, Pakistan*

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S1503TU01

Acarology

Rickettsiae that parasitize ticks and mites

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Rickettsiae are obligate intracellular bacteria that require eukaryotic cells to serve as their host in order to grow. Rickettsiae have historically been placed into three groups based upon serologic and genotypic typing: the typhus group (TG) including Rickettsia prowazekii and Rickettsia typhi; scrub typhus group (STG) including Orientia tsutsugamushi and Orientia chuto; and the larger more diverse spotted fever group (SFG) that comprises over 20 species including the well known pathogens Rickettsia rickettsii, Rickettsia conorii, Rickettsia japonica, Rickettsia africae, etc. While not all rickettsiae have been proven to be pathogenic the list of formally non-pathogenic rickettsiae added to the pathogenic roles has increased over the last several years. In fact, approximately 15 additional tick-borne rickettsioses have been described within the last 20 years alone. This brief review will discuss emerging and reemerging pathogenic and non-pathogenic rickettsiae found in ticks and mites.

Keywords:*Rickettsia, ticks, mites*

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S1503TU02

Acarology

Study of endemic foci of chigger mite as a vector of scrub typhus

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Scrub typhus caused by Orientia tsutsugamushi is an endemic disease in Korea. Chigger mites and field rodents play roles in transmission of the diseases the vector and host of the agent. The purpose of this study is to investigate the density of the chigger mites and field rodents, identification of them and infectivity of the mites with Orientia in Korea. We captured field rodents and collected mites from them between April and November 2009 in fifteen localities in Korean. We captured a number of 78 mice in the central and 62 mice in the southern region and Apodemus agrarius was dominant species in both regions. A total of 9,232 mites were collected from the mice and identified. PCR and sequencing analysis showed that 35 (11.3%) out of the 309 L. scutellare were infected with O. tsutsugamushi, 52 (9.5%) out of the 548 L. pallidum, 8 (11.4%) of the 70 L. orientale, 2 (11.8%) of the 17 E. koreaensis, and 1 (1.1%) of the L. palpale were infected. Phylogenetic analysis showed that strain were Boryong [dominant strain; total number of mites tested (n=1,136); number of positive mites (n=77), 6.8%], Kanda (n=4, 0.4%), Oishi (n=3, 0.3%), Jecheon (n=1, 0.1%), Youngworl (n=1, 0.1%), Wonju (n=1, 0.1%), and not determined (n=17, 1.4%). The density of mites was high at a dry field or a farm and the O. tsutsugamushi infection rate of the mites was high at the site near by a storing reservoir.

Keywords:*scrubtyphus, Orientia tsutsugamushi, mite*

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Tick-borne pathogen surveillance in the country of Georgia: Implications for unrecognized spotted fever group rickettsial disease in humans.

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As part of the Defense Threat Reduction Agency's Cooperative Biological Engagement Program, tick-borne pathogen surveillance and diagnostic training was conducted in the country of Georgia. Ticks were collected using tick drags/flags and from domestic and peridomestic animals during the spring (May-June) and fall (September) seasons. From 2008 to the present, 4,411 ticks were collected and sorted by species and location into 1,236 pools. The pools were screened using real-time PCR assays designed to detect the genus Rickettsia or to detect spotted fever group (SFG) rickettsia. To confirm the identity of the Rickettsia present in the positive pools, multi-locus sequence anaylsis of the ompA, ompB and sca4 genes was completed and confirmed the presence of Rickettsia slovaca, R. raoultii, and R. aeschlimannii. These three species of Rickettsia have been associated with tick-borne lymphadenopathy (also called Dermacentor-borne necrosis erythema lymphadenopathy) disease in humans that ranges from eschar at the tick bite site and associated regional lymphadenopathy, to fever, headache, and rash. Two of these species, R. slovaca and R. aeschlimannii, are known to be transovarially and transstadially transmitted (unknown for R. raoultii) and have been isolated from Dermacentor species and Hyalomma species ticks, respectively, and R. raoultii has been isolated more frequently from D. marginatus and D. reticulatus ticks than R. slovaca. Geographical distributions of the tick species collected in Georgia and their association with SFG rickettsial pathogens will be presented.

Keywords:*vector surveillance, rickettsiosis, ticks, dermacentor, hyalomma, Rickettsia slovaca*

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Acarology

Australian ticks, their rickettsial symbionts and associated human diseases

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Throughout Australia, a number of rickettsiae have been identified within a variety of tick species. The hard ticks Ixodes holocyclus and I. tasmani, which are distributed along the eastern seaboard of the country, have been shown to harbour Rickettsia australis. Recently, we have demonstrated that I. tasmani ticks in Tasmania are carriers of a novel agent, Candidatus R. tasmanensis. In south-eastern Australia, Bothriocroton [Aponomma] hydrosauri, the vector for R. honei, is widely distributed. A subspecies of R. honei (strain marmionii) has been identified in a different tick species, Haemaphysalis novaguineae, found in the north of the country. In Western Australia two tick species, Amblyomma triguttatum and I. antechini, have been shown to harbour the newly identified rickettsial agents Candidatus R. gravesii and Candidatus R. antechini respectively. The soft bodied tick, Argus dewae has also been shown to carry a novel agent, Candidatus R. argusii. This tick has been identified in Victoria and Tasmania but its geographical distribution could be significantly greater. Although we have elucidated several rickettsial species within both soft and hard bodied ticks in Australia, characterisation of these agents is still in its infancy. Human disease has only been associated with three of the species described, namely R. honei and R. honei subspecies marmionii which both cause Flinders Island spotted fever, and R. australis which is responsible for Queensland tick typhus. Further research will allow us to determine the pathogenicity of the Australian rickettsiae and the true association of these agents with human disease.

Keywords:*Rickettsia*

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Ecological changes affecting the distribution and potential for tick-borne diseases in the Republic of Korea

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Over the past century the Korean landscape changed as a result of Japanese occupation, three major wars, and most recently, economic recovery. At the end of the Korean War (1953), the hills and mountains were nearly treeless. A tree planting policy instituted in the 1960s reestablished long-ago lavish forested mountains and hills that make up >70% of the South Korean landscape. Today, these same barren mountains and hills, now covered with 10-50 year-old forests, provide harborage for wild animals, including deer and wild pigs, and birds, increasing the potential for ticks and associated zoonotic pathogens. As tick-borne diseases are not reportable, the impact on civilian and military populations is unknown. However, a recent post-deployment investigation of nearly 9,000 US soldiers deployed to Korea in 1995 showed that approximately 2% seroconverted to SFG Rickettsia, while only 0.2% seroconverted to scrub typhus. In collaboration with Seoul National University and Konkuk University, the office of Force Health Protection and Preventive Medicine, 65th Medical Brigade, has conducted tick-borne disease surveillance (small-large mammals and tick drags/flags) since 2001. Primary ticks collected from small mammals were Ixodes nipponensis, while Haemaphysalis longicornis, H. flava, H. phasiana, and I. pomerantzevi were the primary ticks collected by tick drag. This surveillance resulted in two new records of ticks and the identification of tick-borne encephalitis, Rickettsia, Ehrlichia, Anaplasma, and Bartonella spp. and Borellia burgdorferi.

Keywords:*Ticks, Korea, Haemaphysalis, Ixodes, Environment*

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Ovarian stage and vitellogenin gene expression in diapause adults of the two-spotted spider mite *Tetranychus urticae*

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The most conspicuous aspect of diapause in adult female insects is the discontinuation of oogenesis. In diapause adults, the synthesis of vitellogenin and its mRNA expression are suppressed. However, only a few physiological and molecular studies have been performed on the adult diapause in mites. In the present study, first we histologically observed the ovaries of the diapause females of the two-spotted spider mite Tetranychus urticae and compared them with those of the nondiapause ones. In nondiapause females, transparent oocytes became opaque with yolk deposition between days 0 and 1 in the adult stage at 20ºC. In diapause females, however, ovarian development ceased at a stage having transparent oocytes without yolk deposition; this stage corresponded to the day-0 stage of the nondiapause females. Then we characterized the vitellogenin genes and examined their expression pattern in the diapause and nondiapause females by northern blot analysis. The diapause females did not express vitellogenin mRNA, and therefore, vitellogenesis was indicated to be regulated at the transcriptional level in diapause.

Keywords:*ovarian development, vitellogenin, diapause, spider mite*

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Overwintering ecology of phytoseiid mites in fruit orchards

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In Korea, two species of phytoseiidae are important in the fruit orcahrd mite biological control; Neoseiulus womersleyi and N. californicus. Two species differ in overwintering strategies and also the feeding specialization. The former is a diapausing and the latter is not. From the series of field studies, we developed a method to conserve the phytoseiid population for better conservation biological control by provisioning the sheltered habitat. In this case, providing the food items such as Tetranychus urticae enhanced the field survival of phytoseiids.

Keywords:*Neoseiulus womersleyi, N. californicus, diapause, conservation biological control, environmental to*

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Is solar ultraviolet-B radiation an ultimate factor causing diapause in *Tetranychus urticae* under temperate climatic conditions?

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Diapause in females of Tetranychus urticae is induced by short day length and low temperature, representing the overwintering stage. Previous studies revealed reinforcement of cold hardiness in red diapausing females. However, green non-diapausing females also successfully overwinter on the field host plants in temperate central Japan, suggesting that freezing (or cold) is not evolutional force for diapause induction in such region. Several alternative hypotheses concerning evolutional significance of diapause attributes in arthropods have been suggested, e.g., to control the timing of reproduction, and to survive starvation. Biological impact of solar ultraviolet-B (UVB) radiation on T. urticae has been well documented, and it is probably a major factor for the mites to inhabit lower leaf surfaces. Red diapausing females are known to be invulnerable to UVB radiation in comparison with green non-diapausing females. We studied seasonal change in the deleterious effects of solar UVB radiation, dose-response, and factors in red pigmentation. As a result, the deleterious effects of solar UVB radiation were the most serious in spring. Extent of the biological impact obeyed the Bunsen-Roscoe's law. Eggs and larvae were more vulnerable than adult females. Of the carotenoids, astaxanthin was particularly accumulated in red diapausing females. Feeding was essential for diapausing females to change their body color; probably caused by the accumulation of astaxanthin. Based on these results, we will discuss about the significance of UVB radiation for this mite to maintain diapausing attributes under temperate climatic conditions.

Keywords:*ultraviolet-B, diapause, Tetranychidae, spider mite, carotenoid*

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Virtual reality experiments on a micro-locomotion compensator: seasonal changes in the spectral sensitivity of *Tetranychus urticae*

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Leaves absorb ultraviolet radiation (UV) and visible radiation (VIS), but transmit some of VIS; thus, the undersurface of leaves is less harmful. This may explain the preference of Tetranychus urticae for the undersurface of leaves. To verify this theory, we examined the orientation behavior of T. urticae on a micro-locomotion compensator (MLC). The MLC maintains a freely walking test mite on a glass plate in a constant position by sliding the plate horizontally and logs the positional data in a programmed checkered pattern of light and dark patches. In the light patches, the mite was irradiated with non-directional light by LEDs. Both nondiapausing and diapausing females spent more time in the dark patches than in the UV-lit patches. Nondiapausing females spent more time in the VIS-lit patches than in the dark patches, whereas diapausing females did not show a preference in this regard. Our results show that nondiapausing females avoid UV and are attracted to VIS. These results suggest that nondiapausing females use spatial differences in the distribution of UV and VIS between the two sides of the leaf as a reliable criterion for choosing their habitat. The preference of diapausing females for sunless hibernacula can also be explained on the basis of UV avoidance without any attraction to VIS. Furthermore, the decrease in attraction to VIS accompanying diapause suggests the presence of at least two different photoreceptors each for UV and VIS. We anticipate that this study will provide a basis for examining the seasonal changes in photoreception systems.

Keywords:*behavior, diapause, LED, photoperiodism, UV, wavelength*

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S1505F01

Acarology

Underground ecology of mites in soil porosity and its significance in pest management

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Both applied and basic entomologists are prone to watch only arboreal ecosystems of organisms and to overlook the reality of underground world. Although some ecological knowledge was accumulated, a few solutions have been provided. Problems of soil-inhabiting pests including mites may persist and expand especially under the current trend with more organic materials and less chemicals in agriculture. Understandings of underground ecosystem with biotic and abiotic interactions are urgently needed to establish a sustainable measure of pest management. In the present review paper, the greenhouse spinach mite, Tyrophagus similis, is taken as a model case and current problems and strategic approaches in progress in Japan will be demonstrated to contribute to the development of future pest management. Country-wide distribution of greenhouse spinach damage by mites became evident since the 1990's and a recent increase in use of organic materials and fertilizers (under the government guiding principle) may have promoted further damages. Farmers are facing a difficult task without much support from scientific sectors. An integrated project started in 2010 under a support of Japanese government and scientific approaches were directed form views of biological, chemical, physiological and cultural managements of mites. It is now on the way of establishing a basis of the integrated management system, starting form pest forecasting (by invention of a new watchman trap) to a sustainable control (by biotic and abiotic management of soil environments) of mites in the Black Box.

Keywords:*soil-inhabiting, microfauna, pest management, greenhouse spinach mite, IPM*

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S1505F02

Acarology

Identification of the female sex pheromone and unique fatty acid metabolisms of *Tyrophagus similis* (Acari: Acaridae).

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Tyrophagus similis (hourensou kenaga-konadani in Japanese) is a pest species in horticulture, causing serious damage to spinach, melon, cucumber, pumpkin and maize in green houses and fields (Nakao and Kurosa, 1988). This species reproduces via mating between males and females, and mating pairs are incessantly observable in the stock culture, strongly suggesting the presence of a female sex pheromone in this species. Here, we report a bioassay driven isolation of a female sex pheromone in T. similis, identified as S-(+)-isopiperitenone [(S)-3-methyl-6-isopropenyl-2-cyclohexen-1-one], previously identified as the alarm pheromone of the mite (Kuwahara et al., 1987; Maruno et al., 2012). A number of insect species are able to produce linoleic acid, a fatty acid that was previously believed to be essential for all animals. Here, we investigated unique metabolism of polyunsaturated fatty acids, precursors of prostaglandins and other eicosanoids, in T. smilis.

Keywords:*sex pheromone, fatty acid metabolism*

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Reproductive capacity of greenhouse spinach mite, *Tyrophagus similis* Volgin, feeding on commonly used organic fertilizers

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Greenhouse spinach mite Tyrophagus similis inhabits soil under 5 cm depth. In soil it mainly feeds on organic fertilizers, plant debris fungus, nematodes and microorganisms. For its control, knowledge of its food resources is important. A series of experiments was conducted to investigate reproductive performance of mites while feeding on organic fertilizers in the laboratory conditions. Four fertilizers (Fish lees, Poultry manure, Rapeseed oil cake and Agret©) were provided as fresh food to mites. Nutritional value of fertilizers was observed by means of fecundity of mites for first five days. The results showed that fish lees have higher nutritional value. To know components of each fertilizer responsible for higher fecundity, an addition of amino acids and a variety of fatty acids (saturated and unsaturated) was investigated. The results showed no significant difference of fecundity recorded on coated and uncoated fertilizers with amino acids. In case of addition of fatty acids, the results showed that oleic acid was high, linoleic and stearic acids were moderate, palmitic and linolenic acids were low nutritional values. The promoting or repellent properties were studied by coating extracts of fertilizers on each other. The results showed that no particular repellent effects were found, while fish lees extract has a high promoting effect. To examine nutritional value of fertilizers after used in the field, they were stored for one and three weeks in spinach greenhouse soil and they were provided to mites. The results showed that nutritional value of fertilizers sharply decreased when stored in soil.

Keywords:*Tyrophagus similis, fecundity, organic fertilizers*

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Hydrozetid mites (Oribatida: Hydrozetidae) on water plants in Lake Biwa, Japan, with some notes on its biogeography

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The Hydrozetid mites are usually recorded in Sphagnum bogs in Japan. We investigated an ancient freshwater lake, the 4-million-year-old Lake Biwa in Japan. Hydrozetes parisiensis Grandjean, 1948 was collected from submerged several species of macrophytes by shipboard sampling on two occasions (15th November 2008) in the South Basin of the lake. On this first occasion, at a site six meters deep, the mites occurred within one meter of the water surface. On the second occasion, they were found at several additional sampling points (25th-27th February 2010). This species has previously been known from Europe. In this report, additionally we present the record of H. parisiensis from Lake Biwa and several places in Japan, and together with a clarification of some aspects of its morphology and the sequence of its D3 region of 28S rDNA.

Keywords:*Hydrozetidae, Oribatida, freshwater lake, locality record, Japan, D3 region of 28S rDNA*

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Acarology

Designing digital multi-media identification tools for invasive mites

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Developing and delivering identification aids, keys, and tools to support pest identification requires an important investment in the planning and design phases of a resource to ensure they are valued and used by end-users. Agencies involved with plant protection and quarantine typically have personnel off-shore, at ports, and domestically involved with survey, detection, trade, and identification that require rather diverse types of support based upon levels of expertise and responsibilities. Resource developers should take into consideration the following during the planning and design phases for a future resource: 1) how will the end-user access the resource, 2) what is the level of expertise (i.e., taxonomy, IT, etc.) of the end-users, 3) what type of resource is required for the responsibility (e.g., screening aids, keys, fact sheets, portal, etc.), 4) how and where will the resource will be used (i.e., field, laboratory, identification, verification, diagnostics, surveys, mobile devices, etc.), 5) what type of results are expected by the use of the resource, and 6) what is the scope (i.e., breadth, circumscription) of the resource. Based on numerous years developing and delivering specialized digital multi-media resources for a diversity of teams associated with the United States Department of Agriculture (USDA), the authors will present guidelines for planning and designing valuable, user-friendly, identification resources to support mite identification.

Keywords:*Digital identification web interactive*

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Reproductive performance and acaricide susceptibility of the invading tomato red spider mite, *Tetranychus evansi* Baker & Pritchard

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The tomato red spider mite, Tetranychus evansi, is an invasive herbivore specialized on solanaceous plants, which rapidly spread throughout the world during the last decade. It causes serious damage to crops in Africa and Europe, but has not yet been reported to damage crops in Japan, where it was first found in 2001. To evaluate the potential threat of Tetranychus evansi, life-history parameters, such as rate of development and intrinsic rate of natural increase (rm), for mites collected on seven sites worldwide were assessed on Solanum nigrum. For egg-to-adult development, the lower thermal thresholds (t0) were 11.6-12.0°C and the thermal constants (k) 127-142 degree-days. The rm-values increased with temperature increasing from 15 to 35°C. At 25°C, rm ranged from 0.265 to 0.277 which is high among Tetranychus species. These results indicate that T. evansi has the potential to become a serious pest on solanaceous crops in Japan, just as it has become in Africa and Europe. This study also determined the susceptibility of adults and eggs of nine strains of T. evansi (the seven mentioned earlier plus two more Japanese strains) to 11 acaricides, because chemicals are used as the first line of control when a new pest arises. For all but one acaricides the LC50's on all nine strains were much lower than the recommended concentrations, suggesting they could be used to control T. evansi, should it occur in high numbers in crops.

Keywords:*Tetranychus evansi, reproduction, intrinsic rate of natural increase, developmental performance*

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Acarology

Tomato red spider mite, *Tetranychus evansi* Baker&Pritchard: Invasionand management in Africa

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The tomato red spider mite, Tetranychus evansi, is the most important dry season pest in tomato and indigenous Solanaceae vegetable (nightshades) production in various African countries. The pest probably originates from southern America and spread to Africa, Europe and Asia. It however represents a serious problem mainly in Africa. Several predatory mite species successfully used against other Tetranychus spp. were tested against T. evansi on solanaceous plants, but the results were not promising. However, when T. evansi was reared on plants outside the Solanaceae family, predatory mites like N. californicus were found promising. The pest is believed to sequester toxic compounds from solanaceous plants to defend itself against predators. To control this pest, small scale farmers in Africa rely exclusively on synthetic pesticides, particularly dicofol, dimethoate and lambda-cyhalothrin. However, T. evansi quickly developed resistance against these products. Plant resistance to T. evansi was found in some tomato hybrids and nightshade species. The pest is able to suppress induced plant defense mechanisms. Surveys in parts of South America identified as priority areas by a climatic model, revealed Phytoseiulus longipes (Phytoseiidae), Stethorus tridens (Coccinellidae) and Neozigytes floridana (Entomophthorales) as the most promising biocontrol agents. Phytoseiulus longipes was introduced to Kenya in 2005. A pilot release in 2007 resulted in establishment, although further releases are considered necessary for successful control of the pest. In addition to the natural enemies found in South America, Metarhizium anisopliae ICIPE78 was identified in Kenya as very effective against T. evansi and it is now patented for commercialization.

Keywords:*Tetranychus evansi, IPM, climatic modeling, Predators, Entomopathogenic fungi*

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Expansion and occurrence of *Neoseiulus californicus* (McGregor), a prospective predacious mite species, in Japan and Korea, and the conservation for biological control of spider mites

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Neoseiulus californicus is an effective predator species of spider mites, and has worldwide distribution, including Europe, Africa, and North and South Americas. It had been a minor species in East Asia before the 1980s; however, in Japan, both the abundance and geographical distribution of N. californicus have sharply increased in and after the 1990s. This species was first found in central Japan, but has recently expanded its distribution to the southwest and southern areas. In Korea, the occurrence of N. californicus in the field was first reported in the southernmost island, Jeju Island, in the 2000s. Within the short period, N. californicus has become a dominant species in many fruit orchards with conventional control, such as Japanese pear and citrus. In addition, N. californicus has rapidly replaced the position of Neoseiulus womersleyi, which previously occupied a dominant position among phytoseiid fauna. Thus, N. californicus is considered a new prospective predator species against spider mites in these areas, although the reasons for the increase and expansion remain unclear. As N. californicus populations in East Asia have no diapause capacities, surveys on overwintering sites of this species is important to establish the biological control strategy on perennial crops, such as fruit trees. Most N. californicus overwintered on herbaceous plants with rosette-type leaves on the ground. N. californicus also utilized artificial shelters, such as urethane foam set on the ground in fruit orchards as overwintering sites. Thus, provision of these overwintering sites may be effective for conserving N. californicus populations in orchards.

Keywords:*Neoseiulus californicus, predator, spider mites, fruit tree, biological control, overwinter*

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Acarology

Agricultural acarology in the Philippines: Pest mites and their natural enemies

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The mite fauna of the Philippines is certainly a rich one. In agriculture, many species are recorded as host sampling is expanded. A wide range of phytophagous mites are recorded predominant commercial mite pests in Philippine agriculture. Among these phytophagous mites, spider mites under the family Tetranychidae are the most economically important as they are known pests of both agronomic and horticultural crops. Predatory mites acting on these phytophagous mites are also diverse particularly of importance are the phytoseid mites. Efforts to rear the phytoseid mites are made with some degree of success but efforts are primarily focused on methodology rather than for mass rearing and field releases. Augmentation of these predatory mites in the field is confined to conservation and habitat manipulation.

Keywords:*biological control, Philippines, plant mites*

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Management of *Varroa destructor*, the ecto-parasitic mite of the honey bee using fungal pathogens

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The honey bee, Apis mellifera L., is critical not only for honey production, but also for crop pollination. The added value to U.S. crops from honey bee pollination was estimated at $14.6 billion annually. The ecto-parasitic mite Varroa destructor Anderson and Trueman is currently the most serious pest of honey bee around the world. Bee hives infested with Varroa mite can perish within a few weeks. In addition, Varroa mite has developed resistance to the only two miticides used for control and threatens the honey bee industry and agriculture. Therefore, we examined the use of fungal pathogens as sustainable and environmentally friendly alternative control strategies for Varroa mite. Several fungal isolates were screened for pathogenicity and virulence against Varroa mites. Honey bee colonies with heavy infestations of Varroa mites were treated with different formulations of fungal spores to determine efficacy. Hirsutella thompsonii and Metarhizium anisopliae were found to be highly pathogenic to the mite and harmless to honey bee. Data in the last six years indicated that strip or paddy-blend formulations of M. anisopliae were successful in controlling populations of Varroa mite. We determined that fungal spores (1 x 1010viable spores per gram) with 98% germination and high pathogenicity (95% mite mortality at day 7) provided successful control of mite populations in established honey bee colonies at 10 g of conidia per hive (applied twice). Overall, biological control of Varroa mite is feasible and could be a useful component of an integrated pest management program for the honey bee industry.

Keywords:*Varroa mite, fungal pathogens, environmental friendly, honey bee, pest control*

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Dissemination of exotic tick and tick-borne infectious agents by migratory birds in the United States of America and abroad

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Birds are capable of carrying ticks and, consequently, tick-transmitted microorganisms over great distances and over geographical barriers such as oceans and deserts. Ticks are hosts for several species of spotted fever group rickettsia (SFGR), which can be transmitted to vertebrates during blood meals. In this study, the prevalence of SFGR was examined in ticks infesting migratory songbirds by using Polymerase Chain Reaction (PCR). During the 2009 and 2010 spring migration season, ?2064 northward-migrating passerine songbirds were examined for ticks at Johnson Bayou, Louisiana. A total of 120 ticks were removed from 38 songbird species for tick species identification and SFGR detection. The ticks were identified as Amblyomma longirostre, Amblyomma nodosum, Amblyomma calcaratum, Amblyomma maculatum, Amblyomma spp. and Haemaphysalis species, all originating from South America by comparing their 12SrDNA gene sequence to homologous sequences in GenBank. The nucleotide sequence similarity of the PCR products revealed several exotic SFGR. Our results indicate that exotic tick species arriving attached to migrating songbirds may play an important epidemiological role in importing previously unknown SFGR to the North America.

Keywords:*Ticks, migratory birds, Spotted Fever Group Rickettsia*

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Functional morphology of accessory structures involved in reproduction among gamasid mites (Acari, Anactinotrichida)

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In Mites sperm transfer can be direct or indirect. In direct sperm transfer, males introduce the sperm into the females either via the primary female genital opening (architocospermy and neotocospermy) or via a secondary genital opening (porospermy, podospermy/gonopody, neopodospermy). In indirect sperm transfer, males produce and deposit a spermatophore on the substrate, then, the females collect and transfer it to their genital opening. So contact between the partners is not a prerequisite. Gamasida represent the most species-rich taxon in the anactinotrichid mites and occur world wide. Among gamasid mites only direct sperm transfer occurs but architocopermy, neotocospermy, and neopodospermy have been observed. Several anatomical and functional adaptations are related to these different insemination modes including peculiar accessory structures present both in male and female. In fact in more derived taxa the male chelicerae show peculiar structures, likely adapted to a more precise manipulation of the spermatophores, and related to other peculiar specialized structures in the females and to different insemination modes. Actually, corresponding females show a modified genital system and can be provided with specialized structures to receive the spermatozoa (sperm access system). Moreover, among Gamasida, those taxa provided with modified chelicerae in males show a displacement of the male genital opening in a presternal position to facilitate the transfer of the spermatophore to the chelicerae. These accessory structures enclose very important behavioural, functional and systematic implications still not full explained. Here a review is reported with data regarding the taxa investigated among Gamasida with perspectives for future researches.

Keywords:*gonopods, sperm access system, sperm transfer*

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Anatomy and ultrastructure of the prosomal salivary glands in the water mites *Piona carnea* (Koch, 1836) and *Teutonia cometes* (Koch, 1837) (Acariformes: Hydarchnidia)

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Prosomal salivary glands of the water mites P. carnea and T. cometes were examined both on serial semi-thin sections and in TEM. P. carnea possess four gland pairs, which can be termed medial, intermedial, lateral and ventral, and the medial glands, as in larvae of this species, have unique arrangements one after another on the middle body line. The ducts of the medial and intermedial glands are fused together separately from the ducts of the lateral and ventral glands, which join with the common salivary duct. Both gland ducts are fused and open into the subcheliceral space. Glands in P. carnea are composed of the single very large alveolus built up of the secretory cells arranged around extremely voluminous intra-alveolar cavity (central lumen) and possess similar secretory vacuoles of moderate electron densities, large nuclei and numerous cisterns of rough endoplasmic reticulum. In contrast with P. carnea, adult mites T. cometes have only two pairs of extremely large salivary glands, which may be termed dorsal and ventral. They organize similar to the glands of P. carnea, and their gland ducts fuse together and with the common salivary (podeocephalic) duct, which is finally opened into the subcheliceral space. The cells of the salivary glands of this species are filled with secretory vacuoles of different sizes and densities. Thus, in the adult water mites T. cometes, in comparison with P. carnea, oligomerization of the salivary glands number up to two pairs has apparently occurred.

Keywords:*prosomal salivary glands, anatomy, ultrastructure, water mites*

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Presumed paternal genome loss during embryogenesis of predatory phytoseiid mites

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The reproductive system of predatory phytoseiid mites is thought to be pseudo-arrhenotoky or paternal genome loss (PGL), which would place it between two reproductive systems: bisexual and arrhenotokous reproduction. PGL itself is a remarkable event during embryogenesis. In addition, the reproductive system of phytoseiid mites has interested scientists in terms of its evolutionary significance, and elaborate hypotheses have been developed to clarify phytoseiid reproduction. However, these hypotheses were based on cytological evidence from other animal groups, with insufficient experimental evidence from phytoseiid mites. Therefore, PGL in phytoseiid mites is not confirmed, just presumed. Cytological and histological events during embryogenesis in phytoseiid mites need to be elucidated to supplement the theoretical approaches. In this article, additional histological and genetic evidence of phytoseiid reproduction is presented to better understand the evolutionary significance of paternal genome loss during embryogenesis.

Keywords:*phytoseiid mites, pseudo-arrhenotoky, para-haploidy, paternal genome loss, embryogenesis*

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S1601TH01

Social Insects

Not quite eusocial? There are plenty of fun and merits in studying subsocial insects

Jae Chun Choe
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The evolution of sociality is considered a major transition of evolution. Among the myriads of animals that live solitarily, how and why only the "handful' of animals have reached the levels of sociality is thus a question to be answered. Subsocial insects, in particular, has been a subject of considerable interests, not only because of their "admirable" nature but also for their potential significance in understanding the evolution of eusociality. Two primary evolutionary routes to eusociality have been proposed: the subsocial route and the semisocial route. Due to its inclusive properties of the term 'subsocial', however, many of the examples used to support both the subsocial and semisocial route could fall in the category of subsociality. For the evolution of eusociality, empirical study still lags behind theoretical analysis. Studies of subsocial insects could provide much needed empirical data on both the proximate and ultimate causes of social evolution. In this account, I will try to briefly review the history of the study of sociality in insects, highlight some key issues in the study of subsocial insects, and dare to comment on the recent debate over the importance of kin selection.

Keywords:*Subsocial insects, eusociality, semisocial*

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Social regulation of task-related plasticity in circadian rhythms in honey bee (*Apis mellifera*) workers

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Division of labor in honey bees is associated with plasticity in circadian rhythms. Forager bees (typically > three weeks of age) show strong behavioral and molecular circadian rhythms with higher activity during the day. Younger bees that typically care for ('nurse') the brood are active around the clock with attenuated oscillations in clock gene expression. Genome- wide microarray analysis found 160 and 541 transcripts exhibiting significant sinusoidal oscillation in nurses and foragers, respectively, with peaks of expression distributed throughout the day in both task groups. Nurses that were transferred from the hive to a constant lab environment switch to activity with strong circadian rhythms and onset of activity during the subjective morning. These findings suggest that some pacemakers measure time and regulate circadian processes even in around the clock active nurse bees. We used social manipulations for studying the regulation of plasticity in circadian rhythms. We found that nurse-age bees that were caged on broodless combs inside or outside the hive have strong circadian rhythms in activity and clock gene expression. By contrast, same-age full-sister bees that were caged on brood containing combs were active around the clock with attenuated clock gene oscillations, similar to the pattern typical to nurse bees. Nurse age bees for which we removed the flagella of the antenna, tended the brood like intact nurses, but were more active during the day. Taken together these studies suggest that signals from the brood are perceived by the nurse flagella and regulate plasticity in the circadian system.

Keywords:*honey bee, division of labor, circadian clock, social behavior, genomics*

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Antioxiant and antibacterial activity of different floral honeys from Western Ghats of Karantaka

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Honey has a wide range of therapeutic properties which depend largely on floral sources. In this study six Apis cerana honey samples collected from different locations of Western Ghats of Karnataka were screened for total phenolic content, potential antioxidant activity and antibacterial effects. The antioxidant properties of these samples were assessed by DPPH (2, 2-diphenyl -1-picrylhydrazyl) and ABTS+ radical scavenging activity. The antibacterial tests were done by using gram-positive (Staphylococcus aureus) and gram-negative (Pseudomonas aeruginosa, Klebsiella pneumonia, and Eschiershea coli) bacteria. In addition the pH and color intensity of the honey samples were analyzed. The result of the study demonstrates that, antioxidant activity was low in light color honey and high in dark honey. The color of the honey samples analyzed in this study was variable and ranged from pale yellow to dark brown. All the 6 honey samples did not show the bacterial growth inhibition against Staphylococcus aureus.

Keywords:*ABTS+, DPPH, Eschiershea coli, Klebsiella pneumonia, Pseudomonas aeruginosa*

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The clypeal gland in *Pyramica sauteri*, a novel exocrine structure in ants, and its role in prey capture

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We report on the occurrence of a novel exocrine gland of the dacetine ant Pyramica sauteri. The gland is formed by bicellular class-3 units that open through small pores on the upper side of the peculiar platform-like clypeus. The gland most probably is the origin of the 'labral secretion' that has been reported in literature, and that plays a role in the capture of collembolan prey. Our observations confirm that prey capture involves the stealthy approach of the ant towards a collembolan, which is attracted to the upper clypeal portion of the ant's head, which corresponds with the region where the glandular cells open. When the prey is licking the clypeus of the crouching and motionless worker, it is within the reach of the ant's mandibles and will be seized and carried to the ant nest. Chemical analysis using a SPME-fiber fixed to a micro-injector allows precise small area sampling and shows a similar pattern of hydrocarbons of various regions of the head, but also reveals that the upper clypeal region of foraging ants contains heptadecane and octadecane as two additional compounds, that appear to play a role as prey attractants.

Keywords:*ants, exocrine glands, pheromones, prey capture*

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Termites, the other social insects

Judith Ingrid Dr Korb
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Termites are the second large group of social insects and their complex societies date back more than 130 million years. Unlike the social Hymenoptera, termites find their roots alongside the cockroaches and are basically 'social cockroaches'. This different ancestry is reflected in the organisation of their societies: e.g. Termites are hemibetabolous insects and their workers are larvae, while the workers of the holometabolous social Hymenoptera are adults and the larvae are dependent young. Yet the striking apparent similarity between ants and termites lead to the general assumption that the driving forces of their social evolution were similar. This is debated: The first sterile caste that evolved in termites was the soldier caste which is present in all species (exception: few apical taxa). The role of the workers is less clear. In higher termites (Termitidae) they clearly are an altruistic caste highly engaged in brood care. Yet in the phylogenetically basal lower termites brood care seems to be less developed or absent in wood-dwelling species that spend their entire colony life in a single piece of wood (one-piece nesting termites). Only in taxa that forage outside their nest (foraging species; multiple-pieces nesting termites) brood care becomes prominent. As the wood-dwelling life style is thought to be ancestral in termite's evolution this implies that the forces of social evolution largely differed in termites and social Hymenoptera and that defence played a major role in termites. This has important consequences when applying inclusive fitness theory to worker evolution.

Keywords:*social insects, termite, evolution, soldiers, brood care, behavior*

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S1601TH06

Social Insects

A colony size "sensing" mechanism in an ant

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Colonies of social insects are often regarded as super organisms. Individuals are like cells and organs of a colony. As individual organisms change their characteristics as they grow, colonies of social insects also change their behavior and other traits in response to the colony size. How individual colony members can "sense" their own colony size is, however, generally unknown. In this talk we show the first explicit mechanism of colony size related behavioral regulation. In colonies of the queenless ponerine ant, Diacamma sp. from Japan, a mated worker (gamergate), which effectively monopolizes reproduction as the functional queen, regularly patrols in the nest to inform her presence to other unmated workers and to inhibit their reproduction. Interestingly, she spends longer time in patrolling as the colony becomes larger. We show a simple feedback mechanism, relying on individual contacts, regulates this activity shift in response to changing colony size.

Keywords:*self organization, social physiology, decentralized contol, social insects*

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Provisioning control by females of the earwig, *Anisolabis maritima*: Does she adjust provisioning according to the young's need?

Seizi Suzuki
Hokkaido University, Japan

Provisioning to young is an important form of parental care and is expected to improve young survival and growth. Because the presence of some cost is expected in provisioning by parents, some parents have a flexible investment dependent upon their young's need, to minimize the costs of current reproduction. The evolution of offspring begging signals is predicted based on an evolutionary resolution of this conflict. Anisolabis maritime is a cosmopolitan species of earwig and shows a subsocial behavior where females tend clutches of eggs in soil burrows and progressively bring food to their nest after hatching. I will report on: 1) what signal affects provisioning to young, 2) whether or not flexible control of food provisioning occurs in this species.

Keywords:*provisioning begging Dermaptera*

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S1601TH08

Social Insects

Provisioning call by mothers of a subsocial shield bug

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Offspring of many animal species solicit food from parents using begging signals, while parents manipulate offspring behavior to optimize fitness using various signals. Female Parastrachia japonensis (Heteroptera) provision nests with drupes of the host tree. Provisioning females were recently heard emitting a low-pitched fluttering sound that we characterized in the field using a contact microphone. A single calling event consisted of multiple sound bouts of varying lengths. A fast Fourier transform (FFT) analysis revealed a frequency range of 0~1 kHz. We tentatively designated it 'provisioning call'. Females emitted the call only upon returning to their nymph-containing nests with a drupe. Moreover, nymphs quickly gathered to the drupe while the female was calling. Because nymphs usually hide in crevices throughout the nest debris while the mother is foraging, we suggest that the provisioning call functions to induce nymphs to gather and feed synchronously on newly provisioned drupes. A playback experiment indicated that significantly more nymphs gathered on a drupe with the playback call than without the call. To the best of our knowledge, this is the first report of a parent producing sound and/or vibration signals directly to offspring at repeated progressive provisioning events in a subsocial insect.

Keywords:*call, provisioning, maternal care, shield bugs*

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Mothers regulate synchronous hatching: Maternal vibration in a subsocial burrower bug, *Adomerus rotundus*

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In some social egg-laying species, parents provide special care for embryos at hatching. A subsocial burrower bug, Adomerus rotundus (Heteroptera: Cydnidae) exhibits maternal care, such as defense against predation and providing food. We found that in A. rotundus, mothers displayed a physical vibration (shaking the body rhythmically), while maintaining the egg-guarding posture, ie., holding the egg mass under the thorax, between the forelegs and midlegs. The occurrence of this behaviour apparently had a peak, coinciding with the time when most embryos synchronously started to emerge from their shells. We examined whether A. rotundus mothers induce synchronous hatching by this physical vibration, and tested two hypotheses: that the maternal vibration assists to improve the success of hatching, and that the vibration regulates the hatching schedule. First, we carried out an experiment where the mother was removed just before hatching to investigate the effect of the presence of a mother on hatching rate and hatching synchrony. The control broods, which experienced continuous guarding from the mother, showed a high hatching rate and synchronous hatching. On the other hand, although the experimental broods, which were isolated from the mother, also showed a high hatching rate, they hatched asynchronously. Second, we experimentally exposed mature egg masses to an artificial vibration and monitored the hatching pattern. Synchronous hatching was induced by the artificial vibration. These results strongly suggest that A. rotundus mothers actively regulate synchronous hatching by physical vibration, but they do not assist the embryos in hatching.

Keywords:*Adomerus rotundus, vibration, hatching synchrony, parent-embryo interaction*

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S1601TH10

Social Insects

Use of multiple compass references in path integration of the subsocial burrower bug, *Adomerus rotundus*

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In several species of subsocial heteropterans, the mother bug searches for plant seeds on the ground and provisions them to young in the nest, such as ants do. Therefore, they have acquired remarkable navigation systems to perform this foraging navigation. A female Adomerus rotundus (Heteroptera: Cydnidae) leaves the nest, searching tortuously for a seed around the host plant by walking, and homes directly back when it finds a seed. In this foraging excursion, the female mainly uses a path integration system based on a visual compass referencing canopy gaps. However, canopy gaps seem to be quite unreliable as reference points because they easily disappear from sight by obstacles such as tree trunks and stems and leaves of plants. One potential solution for this problem is that the navigator independently processes multiple compass references beforehand to be prepared for the disappearance. To determine whether the bug independently uses two different visual references in path integration, we let individual females forage in an experimental arena, setting two LED lights from different elevations. We observed the homing behaviour of female bugs when the light was turned off and/or shifted. These experiments revealed that A.rotundus can independently use two different visual points as compass references in path integration. This unique navigational capacity seems to have evolved in order to compensate for the unreliable compass reference in A. rotundus.

Keywords:*canopy orientation, navigation, path integration, subsocial insect, visual compass*

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Subsocial habits and phylogenetic relationships of Neotropical Chrysomelinae (Coleoptera, Chrysomelidae)

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Subsociality, defined narrowly here as the guarding of offspring by a parent may not be a rare condition in Neotropical Chrysomelinae. Recent observations suggest it is an underreported behavior because many taxa are rare in nature, are highly seasonal in reproduction and display tight associations with a small subset of available host plants. We review possible phylogenetic relationships among selected subsocial and solitary taxa living in Central and South America.

Keywords:*subsociality*

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Selection of old nesting sites by *Apis dorsata* in the Northern parts of Thailand

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A total of 27 nesting sites, 14 (52.0%) nesting sites in Chiang Mai and 13 (44.1%) nesting sites in Chiang Rai, the northern parts of Thailand were marked and monthly visited during 2008-2010. The selected nesting sites based on remnants of old combs (a) full comb, (b) half remnant comb, (c) honey comb, and (d) trace of combs were classified into four categories. The results showed that A. dorsata reoccupied four types of nest substrates: buildings (77.8%), water tanks (26.0%), trees (7.4%), and metal frames (7.0%). The full old remnants combs attracted 6 colonies (28.6%), half combs 8 colonies (38.1%), honey combs 3 colonies (14.3%), and traces 4 (19.1%) colonies. In the 2008, 2009, and 2010, 4, 4 and 3 colonies in Chiang Mai and 3, 7 and 5 colonies at Mae Fah Luang University, Chiang Rai reoccupied the same old nesting sites respectively. The average nesting period of A. dorsata colonies in Chiang Mai was 5.5?2.7 months, and in Chiang Rai was 6.2?5.1 months.

Keywords:*Apis dorsata, nesting substrates, reoccupying, old nesting sites*

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Detection technologies of honeybee diseases in Korea

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Honeybee plays a key role in the human and natural world. The economically most important honeybee was formerly considered to be infected by various pathogen. This study describes techniques used to diagnose honeybee diseases. Major detection methods for honeybee diseases include PCR, LAMP, and immunochromatographic assay. The following diseases were diagnosed based on PCR and real-time detection methods: american foulbrood (AFB), european foulbrood (EFB), chalkbrood, stonebrood, nosema, sacbrood virus (SBV), deformed wing virus (DWV), black queen cell virus (BQCV), kashmir bee virus (KBV), chronic bee paralysis virus (CBPV), israel acute paralysis virus (IAPV), Cloudy Wing Virus (CWV). A micro-scale chip-based real-time PCR system based detection method, termed ultra-rapid real-time PCR, was developed to rapidly and quantitatively detect honeybee diseases within 10 min. This method has detected 9 of 12 honeybee diseases mentioned above. Loop-mediated isothermal amplification method (LAMP) has also used to detect 9 of 12 honeybee diseases with high specificity, efficiency, and rapidity under isothermal conditions without expensive equipments. On the other hand, immunochromatographic assay was developed to detect AFB using specific monoclonal antibodies against Paenibacillus larvae spores. Although PCR methods are still being applied at present time, PCR related rapid kits are being developed day by day. Therefore, detection technologies are rapidly being developed, which may facilitate the detection of honeybee diseases as well as monitor for natural infection of Apis mellifera L.

Keywords:*Honeybee, Disease, Diagonosis, Detection.*

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Honeybee and ectoparasitic mite: Modeling approach to understand the complex

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Honeybee is attracting more attention by the public after CCD. Ectoparasitic varroa mite is the most threat to honeybee health. To understanding the complex interaction between honeybee and varroa mite, we tried to model both species dynamics. Mathematical model can provide effective framework for guiding the implementation or simulated studies which sometimes require substantial time, labor and cost. Basic frameworks of the model were employed from the published information. Field monitoring data were used for adjustment of the sub module or validation of the behaviors. Using this model, we provide some scenario for management of the varroa relative to the integrated mite management (IMM) scheme.

Keywords:*Varroa destructor, Apis mellifera, population dynamics, model, acaricide, IMM*

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Current status of managed insect pollinator in Korea

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We investigated the use rate and number of commercial beesfor the pollination of 10 major horticultural crops and fruit trees in Korea. The use rates of bumblebees for 10 major horticultural crops and fruit trees were approximately 7.9% and 2.8% in 2010, respectively. The use numbers of bumblebees as pollinators was more than 64,345 colonies which included 51,400 for 10 major horticultural crops and 12,945 colonies for 10 major fruit trees, in 2009. The use rate of honeybees as a pollinator for 10 major horticultural crops in greenhouses and fruit trees were approximately 48.0% and 7.7%, respectively. The number of hives used for 10 horticultural crops and furit trees was estimated to be 305,216 and 32,386, respectively. The number of honeybees hives used for pollination of 10 major horicultural crops and fruit trees was estimated to be 337,602. The use numbers of honeybees as pollinators was more than 337,602 hives in 10 major horticultural crops and fruit trees, in 2009.The number of honeybee hives used as pollinators of outside crops, including many fruits and vegetables, was estimated to be more than 500,000. The value of honeybees as pollinators was estimated to be more than 8.5% to 15.0% of total Korean beekeeping products. The rate of use of mason bees for 10 major fruit trees was approximately 3.3%. The number of mason bees individuasl used for 10 major furit trees was estimated to be 1,350,000. The value of insect pollinators in 2010 in Korea was estimated at more than $45million.

Keywords:*Insect pollinator, honeybees, bumblebees, mason bees*

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Honeybee visiting and pollinator attracting characteristics of some honey plants as Black locust replacement in Korea

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The apiculture industry was depended on a black locust (Robinia pseudoacacia L.) over 70 percent of total honey production in Korea. The Korean apiculture industry was faced a crisis cause by deterioration of black locust. The deterioration was accelerated by disease and pest increase in the 2000s. The immediate cause of the crisis was disease, pest and anomaly climate but the root cause was absence of honey plant management. Fortunately, nowadays various researches are being carried out to find other honey plants which can use replacement of black locust because there is realized the importance of honey plant as honey resources. The amount of flowering, the number of honeybee visiting and nectar secretion was investigated as main factors in order to find useful honey plant. Korean raisin tree (Hovenia dulcis var. koreana Nakai), linden tree (Tilla amurensis Rupr.) and Korean evodia (Evodia daniellii Hemsl) were selected as the honey plant. In case of honeybee visiting, the number of honeybee visiting on Korean raisin tree were 200 form 12 pm to 13 pm, Black locust were 130 from 9 am to 10 am, Linden tree were 60 from 15 pm to 16 pm and Korean evodia were 40 form 15 pm to 16 pm. In case of nectar secretion, secreted nectar volume of Korean raisin tree, linden tree, Korean evodia and black locust were 4.15$\mu\ell$, 3.58$\mu\ell$, 3.13 $\mu\ell$ and 2.02$\mu\ell$ from a one flower.

Keywords:*Honey Plants, Honeybee visiting, Pollinator Attracting, nectar secretion*

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Analysis of pollen in honey

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This study reports the method of pollen analysis to classify honey samples according to floral sources, in samples from Korea. The pollens in honey have different molecular weights depending upon the honey floral sources. We were purified the pollen in honeys produced from mono-floral and multi-floral honey, respectively. Honey samples used in this study differ due to plant sources, collection periods, each honeys were centrifuged by sucrose density gradient. This study describes a simplified method for determining the total amount of pollen grains and the relative frequencies of pollen from various plant sources in honey. In many commercially obtained honey samples from Korea beekeepers and China store. Pollen of plant species from Prunus sargentii, Castanea crenata var. dulcis, Robinia pseudoacacia and premium grade Honey. The results of this study indicate that pollen in was on different concentration followed by plant source(honey). The data reflects the pollen situation of the honey were particular honey was produced and the identification of plant origin based on the presence of a combination of pollen types of that particular floral source. The pollens in honey have different genotypes upon the plant sources. Analysis of pollen 16s rDNA result showed different amplification form by PCR. The use of sucrose density gradient and PCR combined with pollen identification proved useful in characterizing honey samples from different botanical origins.

Keywords:*Pollen, Honey, Sucrose density gradient centrifugation, Floral source*

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Organic beekeeping and organic honey in Thailand

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Organic honey, is a pure healthy honey from unpolluted virgin forests or from unpolluted organic agricultural orchards, free from pesticides or chemical environmental pollutants. The organic honey in the kingdom of Thailand is new and innovation research since no records in the literatures. Traditional beekeeping in Thailand is possible to be changed to the organic beekeeping by educate the beekeepers. The average annual honey production of a traditional hive is 3-5 kg/annum, while that of a box hive is 5-20 kg/annum. In the country, about 5000 metric tons of honey are produced annually. The types of honeys produced in Thailand are well known according to their seasons and their botanical origins. These types include Eupatorium odortum Dimcarpus fumatus, Hilianthus annus, Sesamum orientale and Croton roxburghii honeys. The prices of these honeys vary from USD 10 to USD 20 per/kg. Longan honey is highly demand in the country and this kind of honey can be exported as the organic honey. The majority of beekeepers extract honey by the traditional straining method, while some beekeepers with modern hives extract their honeys using honey extractors. This method has to be modified to be the standard or organic beekeeping methods. Now in China, organic honey is produced from the Asian honey bee, Apis cerana more than one million colonies in the forest bee flora and the beekeeping practice of Asian honeybees no need to use pesticides and anti-biotics.

Keywords:*Organic beekeeping, organic honey, bee flora, Thailand*

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Study of purified honeybee (*Apis mellifera*. L) venom from Korea

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The aim of the study was to the determination quantitatively of the major constituents of honeybee (Apis mellifera L.) venom from Korea. The chemical composition of honeybee venom from Korea were evaluated. Melittin content varied from 48.5 to 67.2 and averaged 50.7%. Apamin content camewithin a range of 2.0 to 3.2, and averaged 2.8%, and phospholipase A2 content was between 10.8 to 14.5, averaging 12.8%. There is no statistically significant differences for the content of the main component compared standard honeybee venom to domestic honeybee venom. Acne vulgaris is a chronic inflammatory disorder of the sebaceous follicles. The present study was conducted to evaluate the antimicrobial property of honeybee venom (BV) against the etiologic agents of acne vulgaris and the pharmacological activities of BV have been used in wound healing for centuries. Incubation of the skin bacteria P. acnes, clindamycin-resistant P. acnes, Staphylococcus epidermidis or Streptococcus pyrogenes with BV yielded the minimal inhibitory concentration (MIC). To study wound healing, full thickness skin defects were produced on the dorsal area of mice. The wound sizes were small in the BV group compared to the Control and Vaseline groups. The BV group demonstrated decreased TGF-β1, fibronectin and VEGF mRNA levels and increased collagen-I mRNA levels. These data suggested that BV has effective antimicrobial and anti-inflammatory activity against P. acnes and we suggest that BV is an alternative treatment for antibiotic therapy of acne vulgaris and significant wound healing activity.

Keywords:*Honey bee, Bee venom, Apitheraphy*

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RT-PCR analysis of deformed wing virus in the workers of honeybee subspecies *Apis mellifera* carnica

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Deformed wing virus (DWV) is a positive-strand RNA virus that infects European honeybees (Apis mellifera L.) and has been isolated from the brains of aggressive bees in Japan. DWV is known to be transmitted both vertically and horizontally between bees in a colony and can lead to both symptomatic and asymptomatic infections in bees. In environmentally stressful conditions, DWV can contribute to the demise of a honeybee colony. Application of RT-PCR confirmed the presence of DWV infection in the honeybee brain which could adversely affect critical sensory functions and alter normal bee behavior.

Keywords:*Apis mellifera, Deformed wing virus, RT-PCR*

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Declining populations of *Apis cerana* - a threat to agricultural sustainability in Asia

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The Asiatic honeybee Apis cerana is widely distributed throughout Asia, as far north as Japan and China as far west as Iran and Afghanistan and south into India and Sri Lanka. The honeybee Apis cerana which is adapted to the local climatic conditions through centuries of natural selection is threatened and on decline due to changes in land use and intensification of agriculture leading to a loss of wild habitat, reduction in species availability for feeding, and death from pesticides; on the other hand introduction of Apis mellifera into indigenous areas of Apis cerana is encouraging farmers to replace Apis cerana with the exotic bee, and introducing diseases and parasites against which Apis cerana has no resistance. Introduction of disease through exotic species is a widespread problem. The indigenous honeybee species such as Apis cerana, Apis laboriosa, Apis dorsata and Apis florea have been co-existing for centuries without transfer of diseases and parasites. When Apis mellifera was introduced it brought with it European Foul Brood (EFB) and Acarine disease. Other bee species have also been affected. The Apis mellifera parasite Melisococcus plutonius has been detected in colonies of Apis laboriosa, for example. There has also been transfer in the opposite direction: the Apis dorsata parasite Tropilaelaps clareae transferred to Apis mellifera colonies, and was spread by them. The major factor for decline in populations of Apis cerana has been the non-acceptability of this species by beekeepers and the farming community in comparison to Apis mellifera. The details will be discussed.

Keywords:*Apis cerana, decline in bee populations, agricultural sustainability*

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Positional cloning and functional analysis of candidate genes responsible for AcNPV susceptibility in silkworm, *Bombyx mori*

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Baculoviruses were first identified as insect-specific pathogens. Autographa californica nucleopolyhedrovirus (AcNPV) is generally considered to be unable to replicate in domestic silkworm and its cell lines. In 2007, we reported that several silkworm strains including d17 showed remarkable susceptibility to recombinant AcNPV expressing firefly luciferase under the control of the A3 actin promoter. In this study, the susceptible and resistant silkworm strains, d17 and k45, were used for genetic linkage analysis of the AcNPV-susceptible trait. The results indicated that the AcNPV sensitivity is a single gene controlled trait and also a recessive inheritance. By positional cloning, we identify a region tightly linked to this trait to approximately 255.6 kb located on the scaffold Bm_scaf63 on the chromosome 3. Eight candidate gene models were found in the region after searching the silkworm gene model database. These gene candidates were then cloned from the d17, k45 and p50T strains. Sequence analysis indicated that among these eight gene candidates, two genes carried amino acid substitutions. The mRNA expression levels of two genes were significantly increased in the resistant strain k45. To investigate the gene functions related to silkworm's susceptibility to AcNPV, we compared the AcNPV sensitivity of the BmN4 cells, in which the candidate genes were knocked down or over-expressed. The results suggested that there was no significant affection on the AcNPV sensitivity. Consequently, more systematic research is needed to obtain a convincible conclusion of which candidate gene is really involved in AcNPV susceptibility.

Keywords:*Positional cloning, AcNPV, Susceptibility, Bombyx mori, RNAi*

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The host range of *Bombyx mandarina* nucleopolyhedrovirus S2 covers those of *Bombyx mori* and Autographa californica MNPV

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Bombyx mandarina nucleopolyhedrovirus S2 (BomaNPV S2) was isolated from a diseased larva of the wild silkworm, Bombyx mandarina. Notably, BomaNPV S2 exhibits a distinguishing feature that its host range covers those of Bombyx mori NPV (BmNPV) and Autographa californica MNPV (AcMNPV). It can replicate in Bombyx mori (Bm) cells, as well as Spodoptera frugiperda (Sf) cells and Trichoplusia ni (Tn) cells. However, ODVs of BomaNPV S2 in Bm cells contain only one nucleocapsid (SNPV), but one to many nucleocapsids (MNPV) in High Five cells (Tn-5B1-4). The complete genome sequence of BomaNPV S2, as well as its predicted ORFs, was determined and compared to the genome sequence of close relatives. Comparison results showed that most of BomaNPV S2 genome sequence are covered by BmNPV (BmNPV T3 or BomaNPV S1), but several regions seem more similar to those of AcMNPV. This might explain why BomaNPV S2 covers host ranges of BmNPV and AcMNPV. Further experiments of recombined viruses infection demonstrate that gp64 plays an important role in the host range determination of BomaNPV S2.

Keywords:*BomaNPV S2, genome, host range, gp64*

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Control of Baculovirus Infection by host MicroRNA

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Although baculoviruses Autographa californica multiple nucleopolyhedrovirus (AcMNPV) and Bombyx mori nucleopolyhedrovirus (BmNPV) have highly homologous genomic sequences, their host specificity is different. The former can infect insect Sf21 cells, but not silkworm BmN cells. Previous study showed that baculovirus p143 gene plays a key role in host specificity. When the p143 gene of AcMNPV was replaced with that of BmNPV, then AcMNPV was able to infect silkworm. Since the difference in the p143 gene sequences between these two viruses is minimal, three putative miRNAs identified from the silkworm genome that might specifically target to the p143 gene of AcMNPV were studied. Loop-PCR analysis showed that two of these miRNAs were expressed in BmN cells. Further experiments showed that the transfection of synthesized miRNAs, could significantly suppress the expression of p143 gene in Sf21 cells. Furthermore, these miRNAs were able to significantly inhibit the production of AcMNPV in Sf21 cells. Since Drosha and Dicer play crucial roles in the miRNA pathway, siRNAs against Drosha- and Dicer-like genes in BmN cells were used to separately knock-down these two genes. AcMNPV, with its own p143 gene, was then able to infect BmN cells successfully. Therefore, although previous reports have indicated that the differences in amino acid content of P143 may play a role in host specificity, our results suggest that miRNAs generated in different host cells may be more crucial for determining host specificity of an infecting virus to silkworm BmN cells.

Keywords:*AcMNPV, BmNPV, Baculovirus, Dicer, Drosha, miRNA, p143*

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Attenuation of the insect antimicrobial response after baculovirus infection.

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Using genetic information derived from the transcriptome of Spodoptera exigua larvae we designed an expression microarray to decipher the transcriptional response of S. exigua to pathogenic baculovirus infections. Custom microarrays representing around 30,000 unigenes were used to determine the genes differentially expressed in S. exigua Se301 cells challenged with two baculoviruses, a highly specific (SeMNPV) and a generalist virus (AcMNPV). Expression changes were monitored after 4, 12, and 36 hour post infection (h.p.i). Although we observed a fast increase in the number of regulated genes with the progressing infection, host transcriptional shut-down was not observed. Se301 cells responded differentially to SeMNPV and AcMNPV, with more genes being regulated after the challenge with the specific virus. Under our conditions, at 36 h.p.i around 1500 unigenes were commonly regulated in response to both viruses. Among the up-regulated unigenes, we found a few repat family genes, as well as many genes responsible for signal transduction, ATP processing, chitin structure and metabolism and hormonal regulation. Interestingly, among the down-regulated genes we found many unigenes with homology to genes playing different roles in the insect antibacterial response. Given the clear changes in the expression profiles of antibacterial genes as well as other immune related genes we have further investigated the impact of viruses on the intestinal microbiota as well as the viral infection process in the absence and presence of intestinal microbiota. We will discuss the importance of intestinal bacteria in viral infections and propose possible mechanisms of viral regulation of the host immune response

Keywords:*baculovirus, immune, nucleopolyhedrovirus, microbiota, Spodoptera, SEMNPV*

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Functional analysis of apoptosis suppressor genes encoded by *Lymantria dispar* multiple nucleopolyhedrovirus

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Ld652Y cells derived from the gypsy moth, Lymantria dispar, readily undergo apoptosis following infection with various nucleopolyhedroviruses (NPVs), while Ld652Y cells are permissive for productive infection of L. dispar multiple NPV (LdMNPV) without apoptosis induction. LdMNPV encodes two inhibitor of apoptosis proteins (Ld-IAP2 and Ld-IAP3), and we found that neither Ld-IAP2 nor Ld-IAP3 was capable of suppressing apoptosis in S. frugipertda Sf9 cells induced by infection with vAcΔp35, a recombinant Autographa californica MNPV defective in anti-apoptotic gene p35, suggesting that LdMNPV encodes apoptosis suppressor other than IAPs. Thus, we conducted genome-wide screening with LdMNPV cosmid library, and identified a novel LdMNPV apoptosis suppressor gene, apsup, responsible for suppressing apoptosis in vAcΔp35-infected Ld652Y cells. Transient expression assay demonstrated that Apsup also suppressed apoptosis of Ld652Y cells induced by nonviral stimuli such as exposure to actinomycin D and UV. In addition, RNAi-mediated knockdown of apsup expression induced apoptosis in LdMNPV-infected Ld652Y cells. Database search revealed that no characterized Apsup homologues were found, and domain search of Apsup identified no characteristic domains linked to possible functions. To examine the apoptosis suppression mechanism of Apsup, we cloned L. dispar initiator and effector caspases (ld-dronc and ld-caspase-1) which served as the dominant factors in apoptosis signal pathway. Coexpression analysis showed that Apsup suppressed apoptosis in Ld652Y cells by suppressing the activation of Ld-Dronc and/or Ld-Caspase-1. Further analyses of Apsup are in progress to provide insight into virus anti-apoptotic functions against apoptosis-dependent host defenses.

Keywords:*baculovirus, apoptosis, apsup*

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N-glycosylation engineering of baculovirus-silkworm glycoprotein expression system

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Baculovirus expression systems (BES) are popular and widely used to produce glycoproteins because insect cells are capable of transferring N-glycans to recombinant proteins at the same sites as those used for N-glycosylation in mammalian cells. While mammalian cells produce complex type N-glycans containing galactose and sialic acid, insect cells mostly produce tri-mannosylcore or paucimannosidic N-glycans. One of reasons for this difference is the presence of a highly specific N-glycan processing beta-N-acetylglucosaminidase (FDL) in insect cells. Furthermore, insect cells may lack several enzymes required for mammal type N-glycosylation. The difference in N-glycan structure attached to the recombinant proteins might compromise its activity in vivo or induce allergenic reactions in mammals. The BES has several advantages over mammalian protein expression system, therefore much effort has been expended to synthesize mammalian-like N-glycans in insect cells. In this work, we investigated the effect of RNAi-mediated depletion of BmFDL in the N-glycosylation of secretion proteins expressed using BES. We also introduced human alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase (hMGAT2), beta-1,4-galactosyltransferase 1 (hB4GALT1) and beta-galactoside alpha-2,6-sialyltransferase 1 (hST6GAL1) into silkworm cultured cells to provide the enzymes necessary for rebuilding the mammalian-like N-glycosylation pathway. We found that the molecular weights of recombinant glycoproteins were increased by the synergistic effects of FDL suppression and human glycogenes expression.

Keywords:*Bombyx mori, N-glycan, baculovirus expression system*

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Baculovirus expression system as a platform for functional study of novel cellulases

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Wood feeding insects depends heavily on the secretion of a combination of cellulases, mainly endo-glucanases and other glucanases such as exo-glucanases and xylanases, for efficient digestion of the cellulosic materials. To date, although a high number of endo-1,4-beta-glucanases have been found in xytophagous insects, little is known about exo-1,4-beta-glucanases encoded in the genome of these insects. Here we report the identification and isolation of an exo-glucanase, designated as AmCel-5B, from the white spotted longhorn beetle, Anoplophora malasiaca. The insect cellulases and control fungal exo-1,4-beta-glucanase were expressed, characterized in the silkworm-baculovirus expression system and their enzymatic conditions were optimized. Interestingly, AmCel-5B is not only exhibited exo-beta-glucanase activity, but also with obvious endo-beta-glucanase activity. Furthermore, this enzyme is unique in that, although it recognizes Avicel, evidenced as an exo-beta-glucanase, it cannot recognize oligosaccharides smaller than cellohexaose. This may explain why longhorn beetle can well digest hard "living" wood, which contains primarily rigid long fibers. Although it is known that metal ions can enhance the activity of some cellulases, we further demonstrated that reducing agent could work synergistically with metal ions for significant activity enhancement of AmCel-5B. The discovery and investigation of an insect exo-glucanase should lead to a greater understanding of the mechanism for efficient digestion of cellulosic materials by wood feeding insects. The recombinant longhorn cellulases of baculovirus expression in silkworms were found to act synergistically with the commercial cellulases and beta-glucosidase to hydrolyze row agricultural waste. The test formulation can facilitate their potential applications in the production

Keywords:*White spotted longhorn beetle, cellulose, insect exoglucanase, baculovirus, silkworm, and synergism*

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Development of a bi-cistronic Bacmam vector for neural stem/progenitor cells

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Development of a safe and efficient gene therapy vector for neuron-related disease is a critical challenge in neuroscience. Bi-cistronic baculovirus expression vector system (bi-BEVS) is capable to synthesis of high levels of recombinant proteins through one promoter and an internal ribosome entry site (IRES) in insect cell lines for biotechnological applications. Here, we developed a genetic delivery vehicle for production of recombinant proteins based on the bi-BEVS in mammalian cells. A recombinant baculovirus that express DsRed and EGFP protein, under the control of CMV promoter and a chimeric IRES are used to infect rat neural stem/progenitor cells (rNS/PCs). In addition, baculovirus-transduced rNS/PCs show enhanced production of recombinant proteins when used sodium butyrate, an HDAC inhibitor. Our study identifies a nevo approach to express proteins in rNS/PCs, may have beneficial applications in the neurodegeneration therapies.

Keywords:*bi-BEVS, rNS, PCs*

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Cellular responses to baculovirus transduction in human mesenchymal stem cells and induced pluripotent stem cells

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Human mesenchymal stem cells (hMSCs) and mouse induced pluripotent stem cells (iPSCs) can be genetically modified with viral vectors and hold promise as a cell source for regenerative medicine, yet how hMSCs and iPSCs respond to viral vector transduction remains poorly understood, leaving the safety concerns unaddressed. Hereby we explored the responses of hMSCs and iPSCs against an emerging DNA viral vector, baculovirus (BV). First, we uncovered that BV transduction perturbed the transcription of 816 genes associated with 5 signaling pathways in hMSCs. Surprisingly, toll-like receptor-3 (TLR3), a receptor that generally recognizes double-stranded RNA, was apparently upregulated by BV transduction as confirmed by microarray, PCR array, flow cytometry and confocal microscopy. However, we did not find any TLRs activation after BV transduction in iPSCs. Cytokine array data showed that BV transduction of hMSCs triggered robust secretion of IL-6 and IL-8, but not of other inflammatory cytokines. In contrast, BV transduction of iPSCs only elicited mild immune responses. These data demonstrate, for the first time, that a DNA viral vector can activate the TLR3 pathway in hMSCs but not in iPSCs, and lead to a cytokine expression profile distinct from that in immune cells. These findings underscore the importance of evaluating whether TLR3 signaling cascade plays roles in the immune response provoked by other DNA vectors (e.g. adenovirus). Nonetheless, BV transduction barely disturbed the surface marker expression and only induced transient and mild cytokine response, thereby easing the safety concerns of using BV for hMSCs and iPSCs engineering.

Keywords:*baculovirus, mesenchymal stem cells, induced pluripotent stem cells, toll-like receptor, TLR3*

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Development of a baculovirus expression vector for insect cells and mammalian cells

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Baculovirus Expression Vector System is recognized as an important tool for molecular biology and widely used in insect cells and mammalian cells. Development of a shuttle baculovirus expression vector which can function in both the mammalian cell as well as in insect cell would have a significant advantage. Previous studies have demonstrated that the internal ribosome entry site (IRES) derived from 5' untranslated region (5' UTR) of Rhopalosiphum padi virus (RhPV) can mediate cap-independent translation in mammals, plants and insect cells by in vitro translation system. Moreover, earlier studies in our laboratory revealed the presence of cryptic promoter activity in RhPV 5' UTR containing six TAAG motifs in baculovirus infected Sf21 cells. So, series of deletion experiments of the six baculovirus late promoter conservative motifs (TAAG) in the cDNA of RhPV 5' IRES were conducted to identify the specific motif which provide cryptic promoter activity of RhPV 5' IRES. Two tandem repeated TAAG motifs within 309 ~ 418 nts of the 579 nts was responsible for critical promoter activity in baculovirus infected Sf21 cells and the sequence segment was named RP110. Finally, a chimeric sequence was generated by fusing RP110 with cDNA derived 5'UTR of enterovirus 71 to generate an artificial sequence that can function as a promoter in baculovirus infected Sf21 cells and also provide IRES activity in mammalian cells. In conclusion, the chimeric sequence will facilitate the development of a bicistronic baculovirus shuttle expression vector that can express genes in insect cells as well as mammalian cells.

Keywords:*Baculovirus, Rhopalosiphum padi virus, Internal ribosome entry site*

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Sericin as industrial glue and as additive to tissue culture media

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Bombyx mori contains 3 sericin-encoding genes, of which Ser2 generates two mRNAs by alternative splicing. Putative translation products consist of 1740 and 882 amino acids, respectively, and are characterized by 50 repeats of the highly conserved motif DSEKAKPNDRSPSHK (residues conserved in all repeats are underlined) that is remarkably similar to the mussel adhesive plaque protein and to the adhesive trypanosome trans-sialidase. However, the blue mussel protein is rich in Tyr, whereas the sericin 2 proteins contain high proportion of polar residues and are low in Tyr. We examined possible exploitation of recombinant derivatives of sericin 2 in the cell cultures. Most cell types require attachment to a surface and the nature of this substrate has a major effect on cell growth. The performance of many cell types can be dramatically improved by coating dishes with positively charged polymers, (i.e., poly-D-lysine), or tethered adhesion molecules (AMs), including laminin and collagen. We found that crude sericin extracts can spread even over hydrophobic surfaces that cause other water solutes (including poly-D-lysine) to bead up into droplets. We have tested coating of plastic dishes with several natural and recombinant Bombyx sericins for their ability to support growth of Drosophila Cl8+ cells and of the NIL-2 fibroblast-like cells of Syrian hamster. The cells did not grow and mostly perished within 48 hrs in untreated polystyrene plastic dishes, but survived well in dishes coated with truncated recombinant sericin variants (30.2 ? 67.6 kDa) containing the repeats.

Keywords:*silk*

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Display of *Bombyx mori* alcohol dehydrogenases on the *Bacillus subtilis* spore surface

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Alcohol dehydrogenases (ADHs) are oxidoreductases catalyzing the reversible oxidation of alcohols to corresponding aldehydes or ketones accompanied by nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) as coenzyme. ADHs attract major scientific and industrial interest for the evolutionary perspectives, afforded by their wide occurrence in nature, and for their use in industrial synthesis. However, the low activity of ADHs under extremes of pH and temperatur often limits their application. To obtain ADH with high activity, in this study, we used Bombyx mori alcohol dehydrogenases (BmADH) as foreign gene and constructed a recombinant integrative plasmid pJS700-BmADH. This pJS700-BmADH was transformed into Bacillus subtilis by double cross-over and produced an amylase inactivated mutant. The fusion protein containing BmADH was expressed on the spore surface and recognized by BmADH-specific antibody. We also assayed the alcohol dehydrogenase activity of the fusion protein together with the mative BmADH at different pH and temperature levels, which indicated the recombinant enzyme exhibits activity over wider ranges of temperature and pH than its native form, perhaps due to the resistance properties of B. Subtilis spores against adverse conditions.

Keywords:*Bombyx mori, alcohol dehydrogenase, enzymatic activity, Bacillus subtilis spore*

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Positional cloning of the genes responsible for important traits in the silkworm

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The silkworm, Bombyx mori, shows species-specific morphological, physiological and behavioral characters. Some of them have importance in practical breeding and sericulture. For example, the polyphagous feeding is required for the breeding of the strains that adapt to the artificial diet though the normal strains are monophagous to the mulberry leaves. Also recently the strains that produce green cocoons with specific flavonoids are utilized for special silk fabrics or cosmetic materials. After the accurate genomic information was published in 2008, it becomes easier to find the genes responsible for such traits. There are many 'polyphagous' silkworm mutants that feed non-mulberry plant leaves such as the beet and turnip. Recently, we identified two polyphagous genes, spli (soft and pliable) and Bt (Beet feeder). They were allelic mutations of the same gene, Bmacj6, which encoded a putative transcription factor with a POU-homeodomain. These two mutants manifest the abnormalities not only in larval feeding behavior but also in the pheromone preference in the adult males, suggesting that Bmcj6 play multiple roles in the chemical sensing. It is known that the green cocoon trait is controlled by at least three loci, Ga (Green a), Gb, and Gc. We found that a UDP-glucosyl transferase (UGT) gene is responsible for Gb. The normal silkworms do not show the 5-O-glucosyl transferase activity for quercetin, a mulberry-derived flavonoid. On the other hand, the Gb individuals express the enzymatic activity to produce 5-O-glucosylated quercetin. It indicates that the +Gb locus encodes the quercetin 5-O-glucosyl transferase, unreported in other organisms.

Keywords:*polyphagous mutant, mulberry, flavonoids, cocoon color, map-based cloning*

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Responses of *Bombyx mori* cells to nucleopolyhedrovirus infection

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BM-N and BmN-4 cells derived from the silkworm, Bombyx mori, are permissive for B. mori nucleopolyhedrovirus (BmNPV), whereas infection of B. mori cells with other NPVs generally results in various types of abortive infections, providing useful systems for investigating the interactions between NPVs and insect cells. We found that Hyphantria cunea multiple NPV (HycuMNPV) infection of BmN-4 cells results in an abortive infection at the step prior to late gene expression, and that co-infection of BmN-4 cells with BmNPV and HycuMNPV causes restricted BmNPV multiplication, resulting from restricted BmNPV DNA replication and global protein synthesis shutdown of co-infected BmN-4 cells. Analysis demonstrated that HycuMNPV-encoded hycu-ep32 gene is responsible for the restricted BmNPV replication in co-infected BmN-4 cells, showing that the restricted BmNPV replication is rescued in BmN-4 cells co-infected with a hycu-ep32-defective HycuMNPV. Recently, we found that rRNA of BM-N cells undergoes degradation upon infection with various NPVs, including AcMNPV, SeMNPV, HycuMNPV and SpltMNPV. Such rRNA degradation is not observed in other lepidopteran cell lines, Sf9, Ld652Y, Se301 and Splm, suggesting that B. mori has evolved to acquired a unique anti-viral mechanism against NPVs. It was also found that BM-N cells provide advantageous circumstances for BmNPV replication. Our results demonstrated that BmNPV budded viruses cannot be imported into the nucleus of Sf9 and High Five cells which are permissive for AcMNPV, due to lower low-pH-triggered membrane fusion activity of BmNPV GP64, while in BM-N cells, they are successfully migrate into the nucleus through endocytosis and replicate to a high titer.

Keywords:*Silkworm, Bombyx mori, baculovirus, nucleopolyhedrovirus, NPV, infection, interactions*

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Identification and regulation of ecdysone response elements (EcREs) in the *Bombyx mori* cathepsin D promoter

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Bombyx mori Cathepsin D (BmCatD) is specifically expressed in the fat body, and plays a critical role for the programmed cell death of the larval fat body and larval gut during metamorphosis. To better understand the transcriptional control of BmCatD expression, this study was conducted to clarify the regulation of ecdysone response elements (EcREs) in the BmCatD promoter with an AcMNPV (autographa californica nucleopolyhedrovirus) vector containing a Dual-Glo Luciferase Assay System. We identified three putative EcREs located at positions ?109 to ?99, ?836 to ?826 and ?856 to ?846 relative to the transcription start site. Analysis of this EcRE region by overlapping deletion studies showed that the three EcREs suppress the ectopic expression of the BmCatD promoter. These results suggest that the EcREs are necessary for activation of the promoter by 20-hydroxyecdysone (20E) in the larval fat body because EcRE mutations lost the fat body-speci?c expression of the BmCatD gene. Our results further support the crucial role of ecdysone signaling to control cathepsin D gene transcription and they suggest that the heterodimeric complex EcR/USP mediates the activation of ecdysone-dependent BmCatD transcription in the larval fat body of B. mori.

Keywords:*Bombyx mori, Cathepsin D, Promoter, EcREs, Regulation.*

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Apoptosis-related genes involved in cytoplasmic polyhedrosis virus infection in silkworm, *Bombyx mori*

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Bombyx mori cytoplasmic polyhedrosis virus (BmCPV) is a viral pathogen causing enormous damage to sericultural industry. The molecular mechanism of silkworm response against the virus infection is still obscure. In our resent study, some genes related to apoptosis were found to be probably involved in the virus infection in the silkworm. They are Bombyx mori insulin-related peptide-binding protein2 (BmIBP2), ganglioside-induced differentiation associated protein 1 (BmGDAP1), LIM and SH3 protein 1 (BmLASP1), and Synoviolin (BmSYVN). The polypeptides they encoded possessed respectively conserved domains and motifs, with a signal peptide, an IG and an IGc2 domain in BmIBP2, a thioredoxin-like, a glutathione S-transferase-C (GST-C) and a transmembrane domain in BmGDAP1, a LIM, two nebulin repeats and a SH3 domain in BmLASP1, a HRD1 domain in BmSYVN. BmIBP2 mRNA was only expressed in midgut, while others could be found in all the tissues detected. The level of BmGDAP1 mRNA transcripts was higher in midgut and haemolymph than in other detected tissues. That of BmLASP1 was higher in testicle, ovary, head and spiracle than in midgut, fat body, silk gland, vasa mucosa and hemolymph, with the highest expression level in gonad. All of their mRNA expression levels could be remarkably up-regulated by BmCPV challenge except BmLASP1 whose relative expression level in midgut was down-regulated. Western blotting also revealed that BmIBP2 was expressed in midgut in a tissue-specific manner. Based on the results, it is speculated that these genes including BmGDAP1, BmIBP2, BmLASP1 and BmSYVN might be involved in apoptosis immune response against BmCPV infection.

Keywords:*Silkworm, Apoptosis, Gene, Cytoplasmic polyhedrosis virus*

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Molecular and enzymatic characterization of two tetrahydrobiopterin synthesis-related enzymes of the silkworm *Bombyx mori*

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Tetrahydrobiopterin (BH4) plays important role in the production of monoamine neurotransmitters as a cofactor for aromatic amino acid hydroxylases. BH4 deficiency has been associated with many neuropsychological disorders. Oral administration of BH4 has shown improvement for some BH4 deficiency-dependent diseases in human. However, commercial BH4 produced by chemical synthesis pathway are very expensive. Two enzymes are needed in the salvage pathway of BH4 synthesis from sepiapterin (SP), one is SP reductase (SPR) and the other is dihydrofolate reductase (DHFR) in vivo in mammals. SP is a major pigment component contained in the integument of lemon (lem), a silkworm Bombyx mori mutant strain in high concentration, suggesting lem is an invaluable biological resource to extract natural SP. Based on the newly integrated B. mori WGS database and EST libraries, we cloned and identified two genes encoding B. mori SPR (BmSpr) and DHFR (BmDhfr), respectively. Recombinant proteins were expressed using a prokaryotic expression system and their enzymatic properties were analyzed. The results showed that both BmSPR and BmDHFR exhibited high catalytic activity and suitable parameters to substrate. In addition, we have established an effective experimental system to isolate and purify SP from lem silkworms. Together, it is possible and of great significance that SP could be industrially extracted in large scale to bio-pharmaceutically produce BH4 in vitro by co-expressing BmSPR and BmDHFR and using the natural SP as a substrate in the future.

Keywords:*tetrahydrobiopterin, sepiapterin reductase, dihydrofolate reductase, lem silkworm*

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Utilization of the *Bombyx mori* heat shock protein 70 promoter for efficient transgene expression

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For stable germline transformation of Bomyx mori, the promoter of the Bombyx cytoplasmic actin 3 (BmA3) was used to choose to drive the EGFP in piggyBac vector. The BmA3-EGFP marker was found to be useful for the screening of G1 transgenic individuals at the larval stage, but not detectable in embryonic stages. To identify more powerful promoter than previously reported BmA3 promoter, we isolated 9 clones that show stronger signal compared to BmA3 by dot blot hybridization. Among these 9 clones, we focused on one clone which has high amino acid homology (94%) with hsp70 gene of Trichoplusia ni. This clone, named bHsp70 was ubiquitously expressed in all tissues and developmental stage of B. mori, and up-regulated by thermal and ER stress. As result of promoter assay using dual luciferase assay system, we found the highest transcription activity region (-1003/+147) in the 5'-flanking region of bHsp70 gene, which has about 264 fold more intensive promoter activity than BmA3 promoter. Transcriptional activity of bHsp70 promoter under heat shock condition (42℃, 4 hr) was increased over 2 fold than normal condition. Moreover, the bHsp70-EGFP marker was detected ubiquitously in embryonic, larval, pupal, and adult tissues of transgenic silkworm. Therefore, it is possible to screen transgenic individuals before the G1 larval stage using this promoter, because the bHsp70-EGFP marker was expressed in embryos. As above results, we suggest that bHsp70 promoter may be used more effectively for the large-scale production of biologically active recombinant proteins and screening of transgenic silkworm.

Keywords:*Bombyx mori, Heat shock protein 70, Luciferase assay, Promoter*

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Identification of immune-related genes from the japanese oak silkworm, *Anthereae yamami*

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The main targets of our investigation were innate immunity-related genes and effector moleculeS, the key components of humoral defense reaction in the japanese oak silkworm, Anthereae yamami. The expressed sequence tag (EST) analysis was used for screening of genes that are induced to injected bacterial lipopolysaccharide (LPS). Analysis of 1120 cDNAs resulted in identification of 22 novel immune-related genes including pathogen recognition, modulation and putative antimicrobial peptides. In addition, we compared acidic extracted hemolymph protein profiles between control and immune-challenged A. yamami larvae using two-dimensional gel electrophoresis (2-DE). Protein spots showing differential expression were identified by MALDI-TOF and MS/MS spectra. Subsequently, two of them were estimated to be attacin antibacterial peptides with high coverage of sequence. These attacin peptides were intensively induced after an immune-challenge with LPS.

Keywords:*Immune-related gene, antimicrobal peptide, Anthereae yamami, cDNA, attacin*

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Role of *Bombyx mori* prophenoloxidase-activating factor in pupal melanization

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In arthropods, an immune challenge triggers a serine protease cascade that leads to the activation of prophenoloxidase (proPO)-activating factors (PPAFs), which are also called proPO-activating enzymes (PPAEs) or proteinases (PAPs). PPAFs are activated by cleavage between their clip and serine protease domains. Once activated, PPAFs convert proPO to phenoloxidase (PO), which then catalyzes the production of quinones to form melanin. In this study, we identified a Bombyx mori PPAF (BmPPAF) that involves in the pupal melanization. In the fat body, expression of BmPPAF was detected on day 1 to 3 of the pupal stage. RNA interference (RNAi)-mediated BmPPAF knock-down inhibited pupal melanization, resulting in the delay of pupal melanization. Based on these results, we concluded that BmPPAF is involved in the melanization of pupal stage in silkworm metamorphosis.

Keywords:*Bombyx mori, Prophenoloxidase-activating factor, Melanization, RNA interference, Development*

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Structural fundamentals of silk protein biopolymer and biomedical applications of silk nanofibrous scaffolds

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Silk protein has been great interests to materials scientists in biomedical application fields recently because it has excellent processibilities, physical properties and biocompatibility. Many studies and trials are needed to determine the nature of silk structure and mechanism of silk fiber formation in nature. With the progress of tissue engineering technology, tissue engineering scaffolds and implants of silk based biomaterials are paticularly attractive in various forms and silk protein can be a one of very important candidates for biomedical polymers. In the case of silk scaffolds, we demonstrate that highly porous electrospun 3D silk nanofibers can offer exceptional functionalities and benefits over conventional synthetic and natural biopolymers in generating tissue replacement for various cells and organs, specially in the fields of bone and nerve regeneration.

Keywords:*silk protein, scaffold, nanofiber, tissue engineering*

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New biomonitring guidelines using river benthic macroinvertebrates in Japan

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For these 3 years, we are discussing the new guidelines of riverine biomonitoring using macroinvertebrates in a committee of Ministry of Environment, Japan. We are now getting the final version of guidelines. We will introduce these ones for elementary and advanced types of biomonitoring. The former is a nation-wide biomonitoring for students and citizens, which can designate four classes of water quality using 33 riverine animals. Another one is average score method (ASPT-J: average score per taxon) similar to the score proposed by BMWP (Biological Monitoring Working Party in England), which will be used by local institutions, university researchers and students. For the advanced type of biomonitoring, we designated the scores (1 to 10) for 72 families of aquatic insects and other macroinvertebrates. We compared ASPT-J scores and chemical or biochemical (BOD) water quality values using the nation-wide river organism census in Japanese major rivers and streams, and obtained the reference (unpolluted sites) score of ASPT in Japan.

Keywords:*guideline, average score per taxon, reference score,census*

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Change in life history traits of a water strider, *Aquarius paludum* during 1995-2011 in accordance with global warming

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Seasonal records of the last instar nymphs of the water strider, Aquarius paladum in a waterway in Kochi prefecture, Japan (33°00'N to 33°30'N) in 2010 show that populations of new generation adults occur five or more times during the year, from May to December, whereas only three new ones occurred there in 1995. In laboratory experiments, more than 50% of adults are reproductive irrespective of photoperiod. There is a diminishing photoperiodic response in 2007 in relation to induction of diapause when compared to measurements made in previous years (1995-97), suggesting that reproduction may be possible over a much longer period, possibly even in winter, in the near future. In summer of 2008, 2010 and 2011, a high proportion of males sampled had small testes and 60-70% of females had no mature oocytes. This contrasts with earlier summer data, which shows that the testes volume was larger in 1995-1997, and that 75% of females had mature oocytes. These changes may suggest that this population of A. paludum shows partial aestivation. More than 70% of overwintering adults of both sexes are found to have no mature flight muscles in October-November of 2008-2011. A new mosaic-typed wing morph (long fore-wings and short hind-wings) occupied 20% of the overwintering population in 2010. This contrasts with previous observations in 1995 and 2004. The absence of mature flight muscles and the mosaic wing morph in the autumn may be evidence of cessation of dispersal between the summer habitats and overwintering sites on land far from

Keywords:*adult diapause, aestivation, dispersal, photoperiodism, semi-aquatic bug, voltinism*

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The responses and predicted distributions of benthic insects (Ephemeroptera, Odonata, Plecoptera, Coleoptera, and Trichoptera) to global warming: A multivariate study in Korea

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Peninsular environments are one of the ecosystems most vulnerable to global warming. Despite the importance for the conservation of regional biodiversity, they are among the environments least studied with respect to the influences of global warming. In this study, we used benthic macroinvertebrate community data collected on the Korean nationwide scale, to evaluate the potential impact a temperature increase on river ecosystems. Weighted averaging regression models were used to project the relationships between relative benthic insects abundance and water temperature, based on the temperature data of the Intergovernmental Panel on Climate Change (IPCC) A1B scenario. Stoneflies were identified as potentially the most sensitive taxa to global warming, whereas the dragonflies and beetles were benefited from the global warming. The impact of warming on macroinvertebrates was predicted to be minimal prior to the 2060s; however, by the 2080s, species loss of up to 46% was predicted at the highland areas, whereas 11% was predicted at the lowland areas. Potential distribution ranges of cold water species (Plecopteran Sweltsa nikkoensis) in the future decades were expected to decrease continuously over time, while those of warm species (Odonatan Ischnura Asiatica and Coleopteran Psephenoides sp.) were expected to increase from the 2000s to the 2040s and to then decrease until the 2080s. Our projections may be useful for understanding how climate parameters affect the biogeographical patterns of aquatic biodiversity from a thermal-preference perspective.

Keywords:*Global warming, Benthic macroinvertebrates, Aquatic ecosystem, Weighted averaging regression models*

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The current status of Australian Ephemeroptera

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Knowledge of Australian Ephemeroptera is poorly known despite a relative proliferation of work in the last 10-15 years or so. Deficiencies in taxonomic knowledge of the largely endemic fauna have hindered ecological studies particularly the use of mayflies as bioindicators. At present there are over 100 described species with estimations of at least twice that number of species to be described. Further there are presently few researchers working on mayflies, and mayfly taxonomy in particular, in the country. This paper will present the current status and knowledge of Australian mayfly research.

Keywords:*Ephemeroptera, mayflies, Australia*

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Current status of freshwater macroinvertebrates in Korea

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The available literature on the status of Korean freshwater invertebrates was reviewed. Early descriptive and faunistic studies before the 1960s were conducted by Martens (1883-1884) for Mollusca, by Imanish (1940) for Ephemeroptera, and by Cho (1946) for Hemiptera. During the 1960s?1970s, faunistic studies, standing crop comparisons, community studies for water quality assessment, and taxonomic studies on diverse groups of freshwater macroinvertebrates were initiated. Kim (1974) comprehensively studied Korean Trichoptera larvae. During the 1980s?1990s, intensive taxonomic studies of aquatic insects resulted in a monograph (Yoon, 1988) that included 387 species and a key book (Yoon, 1995) that included 340 species. Bae and Yoon (1999) compiled checklist of 638 species of freshwater benthic macroinvertebrates including 491 species of aquatic insects. Since the 1980s, nation-wide government projects such as "National Ecosystems Survey (1986?current: Ministry of Environment, MOE)," "KLTER (2004?current: MOE)," "Korean Native Species Survey (2007?current: NIBR)," "Korean Fora and Fauna Project (2007?current: NIBR), and diverse conservation, restoration, and education programs largely supported the research on freshwater macroinvertebrates in the country. Currently, 1,283 species of freshwater macroinvertebrates including 988 aquatic insects, have been identified in Korea: Cnidaria (1 sp.), Plathyhelminthes (3 spp.), Nematomorpha (8 spp.), Bryozoa (11 spp.), Mollusca (63 spp.), Annelida (52 spp.), Crustacea (42 spp.), Aranea (1 sp.), Ephemeropetra (81 spp.), Odonata (117 spp.), Plecoptera (102 spp.), Hemiptera (83 spp.), Megaloptera (4 spp.), Coleoptera (131 spp.), Hymenoptera (1 sp.), Diptera (334 spp.), Trichoptera (236 spp.), and Lepidoptera (13 spp.).

Keywords:*benthic macroinvertebrates, aquatic insects, freshwater organisms, literature review, taxonomic status, Korea*

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Comparative study on tolerance to sea, brackish and fresh water bodies as habitat in oceanic sea skater of *Halobates micans* and fresh water Halobatinae species *Metrocoris histrio* (Heteroptera)

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Experimental adult specimens ofoceanic sea skater, Halobates micanswere placed in one of 4 salinity conditions [sea water(35-36% salinity), sea water : fresh water = 2:1(23-24%), sea water : fresh water = 1:2(11-13%), fresh water(0%)]8 hours ? 10 days aftercollection from the tropical Indian Ocean (04º09'S, 094º26'E to 10º39'S, 079º18'E). The specimens were checked to see if they were alive without food every 2 hourscontinuously until all adults had died. Paralysis from fresh water shock occurred within 2-9hrs in all specimens after which they died within 2 hours. Significantly longer survival without food was observed in both species on 1/3 and2/3 sea water than on regular sea water. The longer survivalby oceanic sea skaters onbrackish waters may be an adaptation to occasional rain fall on the sea water film.The threshold value for behavioral paralysis to occur due to lower salinity was 9% for H. micans.The average hours of survival of a brackish water species, Metrocoris histrio were 78h, 44h, 11h, 6.5h and 3.5h on 0%, 5%, 10%, 12.5% and 15% salinity water, respectively. Low resistance of this fresh water species to salinity may be related to the lack of experience with brackish water from ecological and evolutionary points of view, while relatively high resistance to lower salinity water by ocean sea skaters may be an adaptation to rainfall on the ocean surface.

Keywords:*Salinity tolerance, oceanic sea skaters, sea water and fresh water species, salinity in habitat*

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Directional movement of aquatic insects in larval and adult stages in streams based on individual based models

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An eco-evolutionary individual-based model (IBM) was developed to accommodate life events of aquatic insects including birth, death, diffusion, and drifting under various biological (e.g., local competition) and environmental (e.g., unidirectional flow) constraints. Evolution of the heritable traits was simulated regarding adapted water velocity, drifting tendency, and upward flight distance by adults. Evolutionary branching in the adapted velocity trait was accordingly addressed as the time progresses, and spatial distribution of assemblages was also presented in riffles and pools. Directional movement such as larval drifting contributed to population maintenance in stream conditions. Upstream flight by adults was also observed to play a key role in adaptive dynamics of insect communities, resolving drift paradox in insect communities in streams. The feasibility of IBM was further discussed regarding monitoring of natural and anthropogenic disturbances under biological and environmental constraints.

Keywords:*stream community evolution, eco-evolutionary processes, adaptive dynamics, drifting, upstream flight*

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Biological and genetic aspects of the non-biting midge Glyptotendipes tokunagai Sasa (Diptera: Chironomidae) during 30 generations of rearing under laboratory conditions

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The non-biting midge Chironomidae has been used worldwide as a water quality indicator and toxicity test organism. This study was conducted to establish a new toxicity test organism by using the chironomid Glyptotendipes tokunagai Sasa, which is widespread in Northeast Asia. Four egg masses of G. tokunagai were collected from the Jungrang Stream in Seoul in April 2007 and were successively reared in the laboratory for over 33 generations in the last 5 years. The number of eggs in an egg mass, hatching rate, and adult body sizes of G. tokunagai were monitored. Genetic analysis was also performed using the partial mitochondrial DNA cytochrome c oxidase I (COI) gene. The influence of temperatures ranging from 8°C to 42°C on development rate was also examined. The number of eggs in an egg mass was shown to rapidly decrease in the early generations, and then it stabilized after the fourth generation ($p < 0.05$). All the examined generations showed a mean hatching rate greater than 75%. Adult body size of the midges was significantly reduced in the initial 3 generations, and then it stabilized in the following generations ($p < 0.05$). The genetic diversity of the reared generations was shown to decrease from 0.004898 to 0.000386. The optimal rearing temperature for G. tokunagai was shown to be within the range of 25?30°C. This is the first case of chironomid domestication in Korea, where the non-biting midge has been successfully reared for at least 5 years under laboratory conditions.

Keywords:*Glyptotendipes tokunagai, successive rearing, cytochrome c oxidase I (COI) gene, toxicity test organism, water quality indicator*

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Species abundance distribution of benthic *Macroinvertebrate* communities under stressful and recovering conditions in the streams

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Benthic macroinvertebrates were monthly collected at 11 sampling sites of the Yangjae Stream, Han River, for three years. Community responses to pollution and recovery were analyzed using species abundance distribution (SAD). The SAD models (e.g., geometric series, log series, and lognormal distribution) reflected pollution impact accordingly. The SADs generally showed geometric series in polluted states, and changed to lognormal distribution as the stream recovered. Variability in community structure was also associated with changes in biological indices (e.g., ASTP, diversity and EPT) and environmental factors (e.g., DO, BOD, conductivity, and pH). The SADs of benthic macroinvertebrate community effectively reflected aquatic ecosystem integrity and could be suitable in monitoring stream communities in stress and recovery.

Keywords:*Community structure, Diversity, Biological integrity, Monitoring, Geometric series, Lognormal distri*

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Local ecological knowledge of the weaver ant *Oecophylla smaragdina*, used as human food, from Vientiane Municipality, Lao PDR

Joost Van Itterbeeck[1], Niane Sivongxay[2], Bounthob Praxaysombath[3], Arnold Van Huis[4]

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A large variety of insect species is used as human food in the Lao PDR. The brood (eggs, larvae, and pupae) of the Asian weaver ant Oecophylla smaragdina (Fabricius), in particular the larvae and pupae of the queen caste, is one of the most popular. The recording of local ecological knowledge of edible insects has so far received little attention. We conducted focus group discussions and participant observations with expert collectors in three rural villages in Xaythani District, Vientiane Municipality, Lao PDR. Our discussions were concentrated on O. smaragdina reproduction, castes, developmental stages, behaviour, nest characteristics and their contents, host trees, and on harvesting methodologies and their effect on the ants. Peoples' local ecological knowledge is partly compatible with the scientific literature on O. smaragdina biology and ecology. Misinterpretations on weaver ant biology and ecology primarily occur due to lack of knowledge on (1) ant behaviour inside undisturbed nests, (2) the role of reproductive forms, and (3) the antagonistic behaviour between O. smaragdina colonies.

Keywords:*Local ecological knowledge, Oecophylla smaragdina, edible insect, Lao PDR*

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Insects - Source of biologically active substances in contemporary pharmacology

Mircea Ciuhrii
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Authors: CIUHRII MIRCEA, MONICA POPESCU Insects, unlike other organisms through various stages of development: egg, larva, pupa and adult. Different chemical composition in each phase. The egg and larva prevails biologically active substances and the transformation of larval stages in pupae and pupae in imago, predominant inhibitory substances (Ciuhrii, 2005,2009, 2010,2011,2012), which served to obtain natural interferon's and cytostatics (06/06/2000 N B. 20605,2011,118115 Ciuhrii patent OSIM) to stop viral infections and tumors. In the near future, insects will cause a revolution in the pharmaceutical industry (Ciuhrii, 2011). In future insects become food sources for humans (Ciuhrii, patent 2011, NA 00963 OSIM Romania). We developed several treatment technologies most dangerous viral diseases and tumors, which in future will allow increased longevity.

Keywords:*SBA, tumors*

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Status of resources insects research and industrialization in China

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Insects are the biggest biological resource on the earth, which have still not been exploited fully. China has more than 5000 years history of utilization of insect resources, for example, silkworm rearing, bee-keeping. In China, insect as resource utilization and commercialization develop rapidly in recent year, resources insects become a special subject in China. Resource insects refer to the insect products or insect body itself or insect behavior have special values or can be directly or indirectly used as resources by humans. Resource insects have economic, ecological and scientific value, deal with insect as industrial raw material, such as, lac insect(shellac), white wax scale insect(insect wax), gallic aphids(tannic acid products), cochineal(pigment), silkworm, honeybee, insect as protein(edible insects), insect as medicine, ornamental insects(butterfly), insects as natural enemies of pests, pollination insects, and the insect cell engineering and so on. Their value is more than 100,000 million. Many research results are constantly emerging, and numerous achievements in scientific research have been put into use. For example, shellac, the secretion of lac insect, has been used as food coatings. 177 species of edible insects were recorded in China and a lot of edible insects are going to restaurant. More than 200 species of medicinal insects were recorded and some of them have being studied for modern pharmacology. And more than 40 species butterflies have reared artificially for butterfly garden and crafts. These insects will provide rich new sources for chemical industry, food, protein products, health care products and medicine.

Keywords:*Resources Insects, Insect commercialization, Insect as industrial raw material, Insect medicine, Insect protein, Ornamental insects*

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Application of butterfly and moth wing patterns on the fabrics? An entrapreunurial approach

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Butterflies and Moths are natural beauties and appear in famous paintings dating back to the days of our cave-dwelling ancestors.They were also focal points in the delicate silk screens of early Chinese and Japanese artists.Butterflies and moths belong to the Order Lepidoptera are covered with thousands of tiny overlapping scales.The prior art does not describe any methodology to obtain this brilliant coloring effects and the patterns displayed by the insects such as butterflies and moths.The basic design was obtained from the good quality digital photos of butterflies and moths.The photographs taken under natural light was used to develop the designs.Preferably high resolution photos of 3456x2596 dimension digital photos were used.The selected butterfly photos were edited using Photoshop to select a portion of the wing design.The selected insect wing design was placed in Page Maker and Corel draw to make designs.Multiple copies of the insect wing design were generated to get full view of the design. It was inferred that multiple designs can be developed from a single species. There is a need for a providing cost effective design and pattern for the textile industry that are very natural with brilliant coloring effect thereby reducing cost and time factor for producing patterns and designs and the present invention provides the same. In the present study the methodology of making designs using wing pattern of moths and butterflies has been tried. An attempt has been made to print those generated designs on fabric.

Keywords:*Butterflies, Moths, Lepidoptera, Wing designs.*

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Utilization of edible insects in China

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Edible insects have been used as a food source for more than three thousand years in China. Recently published articles have suggested that there are more than 200 species of edible insects in China. Edible insects are rich in protein and amino acids, especially eight essential amino acids, which makes them an excellent source of protein. Apart from a good source of protein, edible insects can also provide fat, fatty acids, vitamins and carbohydrates. Edible insects are usually sold in open markets directly as a seasonal and delicious food item in China. Local people believe that edible insects are nutritious and pollution-free food since insects are captured from the wild. Although people increasingly realize the importance of edible insects and gradually accept edible insects as a food source, there are not many products made from edible insects in China. But some researchers and producers have tried to make insect products. Not many of edible insects were evaluated for food safety although researchers developing insect food products understand the significance of food safety and have proposed to develop evaluation procedures. There is no specific law regulating edible insects in term of trading and rearing in China. But there still are some other laws and regulations for animals and plants which may apply to edible insects. If we want to develop edible insects as one food source, some research and practice are necessary. Those are edible insect species identification, large scale rearing, insect food products and insect safety evaluation as food source.

Keywords:*utilization, edible insects, China*

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Assuring food security by using insects as food and feed

Arnold Van Huis
Wageningen University, Netherlands

By 2050, the world will need between 70 and 80% more meat to feed a 9.1 billion population of increasingly demanding consumers. With the amount of available agricultural land being constrained, the production of sufficient protein from livestock, poultry and fish represents a serious challenge for the future. More than 2000 species of insects are eaten worldwide, mainly in developing countries. However, if no action is undertaken this food habit will soon disappear. Edible insects constitute high quality food for humans and feed for livestock, poultry and fish. They are cold blooded and therefore have high food conversion rates. They emit less greenhouse gases and ammonia than conventional livestock. Some insect species can be grown on organic side streams reducing environmental contamination, and transforming waste into high protein feed that can replace increasingly expensive compound feed ingredients such as fish meal, fish oil and soybean. Therefore, edible insects are a serious alternative for conventional production of other animal based protein sources, either for direct human consumption, or indirectly as feedstock. This requires the development of cost-effective automated mass rearing facilities providing a reliable, stable and safe product. In the tropics, sustainable harvesting needs to be assured and rearing practices promoted, while in general the food resource need to be revalorized. In the western world, consumer acceptance can be enhanced by developing processing technologies of insect-derived protein products recipes. The appropriate regulatory framework for allowing insects as food and feed needs serious attention.

Keywords:*food conversion efficiency, entomophagy, greenhouse gas emissions,*

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Structure and properties of silk from African wild silkmoths

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The African wild silkmoths produce commercially important silk and are found distributed along wide range of ecologies in Africa. Variations between silks of four silkmoths; Argema mimosae, Anaphe panda, Gonometa postica and Epiphora bauhiniae, were studied regarding their structure, mechanical and thermal degradation properties using SEM, FTIR, tensile testing and TGA. The wild cocoon shells and fibers showed remarkable differences in surface and cross sectional structures. G. postica and A. panda had thorny spines and hairs on their cocoon surface, while A. mimosae cocoons were highly perforated. Cocoon surfaces had cross bindings, wrinkles and networking of twisting filaments. The FTIR spectra proved the presence of calcium oxalate crystals on G. postica and E. bauhiniae cocoon shells. G. postica and A. mimosae cocoon shells had lower breaking stress and breaking energy than B. mori. G. postica fibers had the highest breaking strain (41.3 %) and the lowest breaking stress (237 MPa). B. mori had the highest breaking stress than the wild silk fibers (427.6 MPa). The TGA curves showed fibers of G. postica and cocoon shells of A. mimosae lost the highest weight due to dehydration (13.3 and 11.9 %, respectively). Total weight losses for degummed fibers and cocoon shells were highest for B. mori and E. bauhiniae (93.4 and 97.2, respectively). The study revealed existing variability in structure and properties among the African wild silk fibers that could be attributed to selection for adaptation and difference in molecular organization and chemical composition of silk spun by each species.

Keywords:*SEM, Stress-Strain, Decomposition, Weight loss, African wild silkmoths*

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Insect biodiversity and humanity

Ke Chung Kim
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Insects are not antagonists but partners to humanity. Today's insects and their cohorts are prominent taxa of long evolutionary history that have been here on the planet long before human species emerged and evolved into "technological being" for the last century. Today's world with over 7 billion people is quite different in many ways under the threat of biodiversity loss and global warming and its climate change from what it was 50 plus years ago which was 3 billion. At this juncture, we no longer can afford to look at insects solely from anthropocentric perspective, as insects explore and settle in new habitats that we create in the context of development for humanity. To develop new application we need to change our approach to studying the biology of insects not as pests or antagonists but as ecological partners in biodiversity, at any spatially defined site which should go a long way for science. Insect biodiversity refers to the totality of everything about the insects on the planet which our knowledge base is rather incomplete and scanty as yet. We must advance our research on diverse aspects of insect biodiversity in order to expand the application and development of insects for humanity which help to advance sustainability of insects and their cohorts whereas human sustainability may be warranted for perpetuity.

Keywords:*Insect Biodiversity*

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Arazyme and highly active enzymes developed by microbes from insects in Korea

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Korea Research Institute of Bioscience and Biotechnology, Republic of Korea

Arazyme, a highly active protease was developed by the microbe isolated from the spider, Nephilaclavata in Korea. Arazyme has high enzyme activityrelatively in the wide range of temperature, pH, substrates, and salt concentration. Arazyme has also natural antibiotic and anti-inflammatory activities. Arazyme is using commercially inthe field of food, animal feed, cosmetics, life cares, laundry detergent, leather processing, and pharmaceuticals. Other highly active enzymes, such as xylanase, mannanase and lipase, were developed from the beetle etc. These enzymes are using especially for animal feed to increase digestion rate and to prevent diseases of animal. And these enzymes are also using for biofuel production industry.Insect Biotech is the leading company which has commercialized the highly active enzymes from insects in diverse industries.

Keywords:*Arazyme, Nephilaclavata*

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International entomology

Thomas Albert Miller
Entomology, University of California, Riverside, U.S.A.

The USA recently celebrated the 150th anniversary of the Morrill Act considered by many to be one of the most successful laws ever passed. It established the land grant universities making higher education available to everyone. It was followed by the Hatch act a few years later that established the Agricultural Experiment Stations in the USA. These two events opened up new fields, but expanded the search for science and technology as underlying innovation. Many generations of students from abroad benefitted from training in these centers. Some stayed to become faculty members and others returned to their countries of origin to create a science based support for agricultural research. It is this network of scientists who form the backbone of international entomology. The tools available for dealing with crop pests and diseases can be counted on the fingers of one hand. This leaves few choices when faced with new threats. Modern application of genetically modified crops and insects have come under unprecedented scrutiny. Resulting in new tools being blocked or abandoned. It is difficult to explain why this has happened, but that reality will not go away. The two main examples beyond transgenic plants are transgenic insects and symbiotic control of crop pests and diseases. These examples and the reasons for preventing approval will be discussed.

Keywords:*International Entomology*

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Molecular mechanism and application of coprisin, defensin-like peptide, from copris tripartitus

Jae-Sam Hwang[1], Eun-Young Yun[2], Joon-Ha Lee[3], Ho Kim[4], Dong Gun Lee[5]

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We report the isolation and characterization of a novel defensin-like peptide from the dung-beetle Copris tripartitus. A cDNA encoding an 80-mer defensin-like propeptide was identified and its predicted molecular mass and pI were 8.6 kDa and 8.7, respectively. A 43-mer mature peptide was also selected and named herein as Coprisin. We also investigated active sequences of Coprisin by using amino acid modification. The antimicrobial activity of chemically synthesized coprisin against human bacterial pathogens was subsequently investigated. Coprisin exerted antifungal and fungicidal activity without any hemolytic effect. Confocal microscopy indicated that coprisin accumulated in the nucleus of cells. The membrane studies, 1,6-diphenyl-1,3,5-hexatriene, calcein-leakage, and giant unilamellar vesicle assays, confirmed that coprisin did not disrupt the fungal plasma membrane at all. And we examined whether treatment with a coprisin analogue (a disulfide dimer of the nine peptides) prevented inflammation and mucosal damage in a mouse model of acute gut inflammation established by administration of antibiotics followed by C. difficile infection. In this model, coprisin treatment significantly ameliorated body weight decreases, improved the survival rate, and decreased mucosal damage and proinflammatory cytokine production.
A coprisin analogue peptide selectively caused a marked decrease in cell viability in Jurkat T, U937, and AML-2 cells (human leukemia cells), but was not cytotoxic to Caki or Hela cells. Fragmentation of DNA, a marker of apoptosis, was also confirmed in the leukemia cell lines, but not in the other cells. In conclusion, our results demonstrate coprisin as a potential therapeutic agent, possibly can be replaced traditional antibiotics to overcome antibiotic resistance.

Keywords:*Copris tripartitus, defensin-like peptide, coprisin*

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Clarifying Initiatives for Retaining Women Entomologists in Academia

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A recent survey of Entomology Departments in the U.S. from 2007-2010 showed that the number of female Ph.D. students increased from 253 to 265 (43 to 48%), and the number of male Ph.D. students decreased from 342 to 386. During that same time, the number of female faculty members increased from 95 to 104 (from 17 to 18%), and the number of male faculty members decreased from 476 to 464. The number of female Assistant Professors decreased (29 to 22%); Associate Professors increased (28 to 31%); and Professors increased (8 to 12%). Twelve causes for concern identified in a 2011 American Physical Society study will be discussed. It is critical to establish an environment and culture that empowers women and men to succeed in academia, and goes beyond bias and barriers. A Call to Action is proposed, focusing on the campus framework for monitoring progress, analysis and utilization of data for organization change, and commitment to take corrective action.

Keywords:*Women Entomologists*

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Family and scientific career

Silvia Dorn
ETH Zurich, Switzerland

Combining family and scientific career remains a challenge for women scientists. I will outline how family and career simultaneously evolved in my case. Helpful perspectives include awareness of the different durations of the time periods that are associated with major obligations for career, marriage and family. They also include awareness of choices for one, two or all three of these options, and their respective consequences. Combining all three options means that time becomes one of the most valuable resources in the life of the woman concerned. Setting priorities accordingly might require one, for example, to renounce some hobbies. Organization of private life and useful approaches will be discussed, as well as unexpected reactions from the professional environment. Mastering the combination of professional life and family is a sign of strength, but others may perceive it as a mere risk. Overcoming such prejudices takes time and energy. Leaving the isolation to obtain support and inspiration from other women scientists is a promising step to take. Opportunities for improvements at the institutional and global level will be addressed. They include spontaneous networks and formalized associations. Further, recognition of great achievements of women scientists is a positive signal that is understood globally. Invited submission: Women in Entomology ? Challenges and Achievements in a New Era (19-1.)

Keywords:*Science, women, behavior, career*

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Taking the "Men" out of "Mentoring"

Ernest S. Delfosse
Michigan State University, U.S.A.

Effective mentoring is a difficult, learned skill; cross-gender mentoring can be even more challenging. Men are often placed in the position of mentoring women, but do not always understand what needs to be done or exactly how to do it; the same can be said for men mentoring men, women mentoring women, or women mentoring men.Cross-gender mentoring works best when in a safe mentoring environment for mentor and mentee, coupled with an understanding of what is needed during the period of mentoring, and taking into account cultural and gender differences. Cultural and gender assumptions may inadvertently affect the success and perception of mentoring. Basically, the role of a mentor is to provide or transfer something of value to the mentee at a critical time in the mentee's career development. In this presentation, I will discuss these issues from the perspective of a man who has mentored women in academia and government.

Keywords:*cross-gender mentoring*

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ICE 2008 - A glimpse of the inside story

Shirley Hanrahan
University of the Witwatersrand, South Africa

I hope to share the feeling of the roller coaster experience of committing to an undertaking like ICE2008. This talk explores some of the behind the scenes activities, responsibilities and organization needed for a successful international entomology congress. These are completely invisible to the average congress delegate. It will deal with aspects of the pre-bid preliminaries, bid funding, congress funding and financial management. Communications, both with the large number of entomologists who contribute to the congress organization and scientific programme and at a smaller scale the executive committee, play a vital role in making things function smoothly. How this was done? Choosing a professional congress organizer company that is experienced, knowledgeable and professional, but most important of all compatible with the aims and focus of the congress, is vital. This talk also serves as a tribute to a small entomological society that backed their first woman president to the hilt to make this venture a success.

Keywords:*ICE 2008*

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Lessons from symbiotic theory for navigating, surviving, and succeeding in an academic career in entomology

Diana L. Six
University of Montana, U.S.A.

Symbiosis denotes close interactions between organisms of different taxa. These interactions can be categorized as neutralism, commensalism, mutualism, antagonism, or parasitism depending upon outcomes, which in turn, may be context dependent. As an entomologist with a passion for studying symbioses between insects and microorganisms, I have often been struck by the parallels between symbiosis and my professional relationships in academia. The interaction type that occurs between 'professional taxa' can help or hinder the success or survival of one or both 'organisms' involved. Interactions between myself and mentors, administrators, collaborators, and students will be used to illustrate use of symbiotic theory to navigate the symbiotic web of the university system. I will discuss how to maximize mutualisms, stop wasting time on (some) commensalisms, and avoid or negate parasitism. Adaptive approaches include cost-benefit analyses and increased up-regulation of recognition mechanisms. I will also discuss how an NSF ADVANCE program, PACE, helped transform opportunities (and change symbiotic outcomes) for women in science at my university.

Keywords:*symbiosis, NSF ADVANCE, entomology, academic career*

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The 7 habits of a highly successful family friendly mentor

Genet M. Tulgetske
University of California, U.S.A.

Several recent studies agree that effective mentorship is crucial to the success of women in science. For many women, the ability to be "family friendly" is a major contributor to effective mentoring. In fact, NSF recently recognized the need for more family friendly policies to increase participation and retention of women in science. In this talk, I will describe seven habits of a family friendly mentor from the perspective of a recent PhD, outlining the characteristics women and non traditional graduate students and postdocs seek most in an effective mentor. I hope, through this talk, to inspire young/early career entomologists to actively seek mentoring that will enhance their academic and professional experiences and to encourage established researchers to provide family friendly mentoring to women and non traditional students and postdocs attempting to juggle families and achieve successful careers in entomology.

Keywords:*family friendly*

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O101M01
Taxonomy

The Red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), an entomological 'elephant in the room'

Paul Rugman-Jones[1], Christina Hoddle[2], Mark Hoddle[3], Richard Stouthamer[4]

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Elephant-like in size and form, the red palm weevil (RPW), Rhynchophorus ferrugineus, is widely considered the most damaging insect pest of palms in the world. Originally native to southern Asia and Melanesia, RPW has invaded much of the Middle East and Mediterranean basin in recent decades, and now poses a serious threat to commercial and landscape plantings of palms in North America. Despite its large size (20-40 mm), confusion remains over the specific status of this pest. Coloration of adult RPW is extremely variable, ranging from predominantly reddish-brown to almost black but with a distinct red stripe. This has historically led to the classification of color-defined variants as distinct species. However, based on morphological, molecular-genetic and breeding data, RPW is currently considered a single species. Using sequences of mitochondrial and nuclear DNA, we re-examined genetic variation among RPW specimens collected throughout its native and invaded range and found compelling evidence that RPW is in fact a complex of two species: one widespread and highly invasive; and, one with a relatively limited known distribution. The latter species recently showed up in California and may have gone undetected in other areas. Accurate identification is likely to be critical to the successful development and implementation of control strategies for these two species, and this 'elephant in the room' can no longer go unaddressed.

Keywords:*cryptic species, COI, rDNA, molecular identification*

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O101M02
Taxonomy

O101

Click beetles (Coleoptera: Elateridae) of Maharashtra: A case study for Indian Elateridae

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Maharashtra is the third largest state of India. On the western side lies north Western Ghats and Konkan. Central part of the state is semi arid while eastern part has dry deciduous forest. Out of the 475 species of Elaterida of India, as recorded in literature, 52 species under 26 genera were reported from different parts of Maharashtra. Specimens were collected from different regions of the state. The specimens were brought to the laboratory and kept alive in the terrarium as far as possible. They were fed with different types of food. A preliminary study of the jump with respect to the morphometry was carried out. The dead specimens were preserved in 70% alcohol. They were studied for their taxonomy.

Keywords:*Elateridae, Taxonomy, Western Ghats, Maharashtra, India*

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O101M03

Taxonomy

O101

Mitochondrial DNA phylogeoraphy of Japanese population of *Phelotrupes auratus* (Coleoptera, Geotrupidae) and implications for conservation

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In biological conservation, taxonomic classification considering evolutionary significant units is important. Phelotrupes auratus (Motschulsky), a coprophagous beetle distributed throughout most of the Japanese archipelago, is listed in the Red Data Book of Japan as being near threatened in several prefectures of the country. Given the current conservation status of this species, knowing the precise taxonomic and phylogeographic characteristics of P. auratus populations is important for the conservation of this species. Partial sequence data of the mitochondrial cytochrome c oxidase subunit I gene (745 bp) of 57 P. auratus specimens from around Japan were compared with a subspecies classification based on mainly geographic color variation. A total of 42 haplotypes were identified and phylogenetic trees inferred using maximum parsimony, neighbor joining, and Bayesian inference methods were highly congruent. The reconstructed phylogenies suggested that the P. auratus populations could be separated into two distinct lineages: Group A consisted of 35 haplotypes from the islands of Honshu, Shikoku, and Hokkaido, and Group B consisted of seven haplotypes from the islands of Kyushu and Yakushima. Group A could further be subdivided into sublineages A-1 and A-2, which consisted of 11 haplotypes from eastern Honshu and Hokkaido and 10 haplotypes from western Honshu and Shikoku, respectively. The average genetic distances within Group A were positively correlated to the geographic distance between sampling localities. While the phylogenetic relationships among haplotypes did not correspond with the subspecies classification based mainly on geographic color variation, the findings suggest that these two lineages need to be conserved separately.

Keywords:*subspecies classification, cytochrome c oxidase subunit I (COI) gene, dung beetle*

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O101M04
Taxonomy
O101

Comparative embryology of adephagan beetles: Its phylogenetic implications

Yukimasa Kobayashi
Tokyo Metropolitan University, Japan

The embryological features of three adephagans, Dineutus mellyi (Gyrinidae), Carabus insulicola (Carabidae), and Hydaticus pacificus (Dytiscidae), were discussed with emphasis their phylogenetic implications. In Dineutus, ten pairs of tracheal gills arise in the abdominal pleura which originate in subcoxae; thus, our observation denies the interpretation that the tracheal gills are homologous with thoracic legs. In Carabus, despite the absence of larval abdominal legs, two pairs of appendage-like swellings, the medial and lateral ones, are tentatively formed on both sides of each of the first eight abdominal segments. The medial swellings in the first abdominal segment develop into pleuropodia. Those in other segments are serially homologous with pleuropodia, but they become flattened and form the larval hypopleura. The lateral swellings, which soon disappear, arise in the subcoxal parts of the medial swellings. The subcoxal parts become the larval abdominal epipleura; thus, lateral swellings can be regarded as temporal subcoxal projections. Considering the subcoxal origin of the tracheal gills in the aquatic Dineutus, the lateral swellings of the terrestrial Carabus might be the vestiges of tracheal gills. In Hydaticus, no appendage-like projections other than pleuropodia appear in the abdominal segments. In both Dineutus and Carabus, the last (10th) larval abdominal segment is formed by fusion of the embryonic 10th and 11th segments, whereas in Hydaticus, the last (9th) segment is formed by fusion of the embryonic 9th and 10th segments (11th segment is highly reduced). These findings suggest the higher position of the Dytiscidae in Adephaga and the aquatic origin of Adephaga.

Keywords:*Dineutus, Carabus, Hydaticus, embryo, phylogeny, subcoxa, tracheal gill, pleuropodium*

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O101M05

Taxonomy

O101

First morphology-based phylogeny of the Saprininae subfamily (Coleoptera:Histeridae) shows remarkable shifts in their biologies.

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Phylogenetic analyses were performed based on the type species of each sub(genus) of the Saprininae occurring around the world, with exclusion of three taxa that were not available. 11 taxa were also included to test the monophyly of the Saprininae. The total number of in-group taxa was 72. In total, 95 morphological characters of adults were scored and analysed to determine the relationships between the (sub)genera of the Saprininae . Cladograms were rooted with exemplars of Dendrophilinae, Bacaniini, Abraeinae and Anapleini. Results confirm the monophyly of the subfamily supported by an autapomorphy (presence of sensory structures of antenna, unique among the Histeridae), as well as other weaker autapomorphies. Basal-most taxa of the subfamily are mostly inquilinous or myrmecophilous, however, inquiliny itself is estimated to have evolved at least five times and myrmecophily three times independently within the Saprininae . Termitoxeny has evolved two times independently within the Saprininae . Most derived taxa of the Saprininae are psammophilous, and psammophily is thought to have evolved once and has been subsequently lost several times. Ecological adaptation for life in caves (troglophily) has likewise evolved two times independently. Because of the low resolution of the cladogram, no major changes in the Saprininae classification are proposed and three informal groups of taxa are instead designated as pointers for the future studies. Additional evidence is needed for a reliable placement of the aforementioned 'groups' as well as for the clarifying the inter-relationships of the heterogeneous and ill-defined genera.

Keywords:*Coleoptera, Histeridae, Saprininae, Phylogeny, Biology*

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O101M06

Taxonomy

O101

Genetic differentiation and phylogeography of *Neolucanus* (Coleoptera: Lucanidae) in Taiwan

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Neolucanus stag beetles distributed in Oriental and Paleoarctic regions, and five known species involving four subspecies were recorded in Taiwan. Taxonomic debates among N. swinhoei Bates, N. eugeniae Bomans, N. doro doro Mizunuma, and N. doro horaguchii Nagai had lasted for a long time. It is generally believed that these diurnal species with overlapping morphological characters differentiated in different mountain areas was owing to weak flight capability and short adult stage. To clarify taxonomic status of these stag beetles, we compared shape of canthus, number of serrate mandible teeth, elytra coloration, elytra luster, and molecular characteristics, i.e, COI, 16S rDNA, 28S rDNA, and wingless, between populations. Morphometric analysis showed two groups of N. swinhoei and the others. However, phylogenetic tree combined COI and 16S rDNA using Bayesian and MP inferences showed four major lineages; and no or low variation was found in 28S rDNA and wingless. Intermingling DNA sequences from different populations in each lineage indicated the possibility of hybridization or introgression event between these species. Results in this study did not completely consistent with the previous taxonomic studies. Weak flight capability and short adult stage reducing gene flow possibility between populations may accumulate variations in local areas. Furthermore, we believed multiple refugia and/or invasions have been occurred in these stag beetles during Pleistocene, which would affect their current distribution and lead to ambiguous in taxonomic recognition.

Keywords:*Neolucanus, morphometric analysis, phylogeography, genetic differentiation*

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O101M07

Taxonomy

O101

Phylogenetic position of termitophilous rove beetle tribe Termitohospitini (Coleoptera: Staphylinidae).

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Rove beetles (Coleoptera: Staphylinidae) are the most ecologically and morphologically diverse insect guests of termite colonies (so-called "termitophiles"). More than 650 species are known from the subfamily Aleocharinae; indeed, there are 12 aleocharine tribes composed exclusively of termitophilous species. One such tribe is Termitohospitini. The species in this tribe exhibit high morphological diversity, with the body form varying from limuloid to physogastric. Two species, Sinophilus yukoae and Japanophilus hojoi are known to have a distinctive sensory patch on segment III of the maxillary palpus. Although this structure had not been found in other species of Aleocharinae, we have now discovered it in other Termitohospitini species, as well as in the genus Myllaena (tribe Myllaenini). We therefore investigated the phylogenetic relationship between Termitohospitini and Myllaenini. This analysis revealed that the Myllaenini formed a monophyletic group with Termitohospitini and Masuriini. The sister group of this clade is estimated to be Pronomaeini, which previously had been considered close to Myllaenini. This topology is supported strongly by mouthpart characters, and we therefore synonymize Termitohospitini and Masuriini with Myllaenini. Based on host termite records, we hypothesize that Termitohospitini may have originated in the Neotropical region, and spread to the Oriental region where the tribe evolved its autapomorphic characters. Our results indicate that the present organization of termitophilous aleocharine systematics may tend to overemphasize the unique characters of each group.

Keywords:*Phylogeny, termitophile, Termitohospitini, Myllaenini, Masuriini, Baysian analysis, synonymy.*

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O101M08

Taxonomy

O101

Phylogeny and biology of *Ischyrosonychini* Chapuis 1875 (Coleoptera, Chrysomelidae, Cassidinae)

Choru Shin
University of Kansas, U.S.A.

Leaf beetles (Coleoptera: Chrysomelidae) form one of the largest beetle families, with most members feeding on living plants. Members of the subfamily Cassidinae often show very colorful patterns, diverse morphology, and many interesting behaviors. The New World tribe Ischyrosonychini Chapuis includes seven genera and 66 species. It is divided into three distinct groups (former tribes Asterizini+Ischyrosonychini+Physonotini). However, synapomorphies uniting these three groups were previously unknown. I conducted a phylogenetic analysis on Cassidinae s. str. to address this problem. A stridulatory file on the vertex in both male and female adults was discovered to represent a synapomorphy for the tribe; stridulation using these files was recorded. The genera Asteriza Chevrolat, Eurypedus Gistel, and Cistudinella Champion are shown to be reciprocally monophyletic and the "physonotine" genera (Enagria Spaeth, Eurypepla Boheman, Physonota Boheman, Platycycla Boheman) are shown to represent a paraphyletic group. Detailed morphological and biological studies were conducted. Adult color change in Eurypepla calochroma (Blake) was observed and a detailed study of the structure of the pronotum and elytra was conducted by histological sectioning. Larval urogomphi are discussed and a distribution map is provided.

Keywords:*Chrysomelidae, Cassidinae, Ischyrosonychini, Phylogeny, Stridulation, leaf beetle, New World.*

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O101M09

Taxonomy

O101

Revision of the *Dorcus velutinus* species group (Coleoptera: Lucanidae) through integrative taxonomic approach

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The Dorcus velutinus species group consists of six species and one subspecies described to date: Dorcus velutinus Thomson, D. ursulus Arrow, D. taiwanicus Nakane et Makino, D. tenuihirsutus Kim, D. japonicus Nakane et Makino, D. carinulatus carinulatus Nagel, and D. carinulatus koreanus (Jang et Kawai). To examine the species entity and their phylogenetic relationships, both molecular analysis using 16S rRNA partial sequences of mtDNA and morphological examination were performed. For the morphological study, the following type specimens were examined: Gnaphaloryx cinereus, D. ursulus, D. taiwanicus and D. japonicus. As a result, we not only recognized distinct genetic divergences among all existing species but also infer that D. velutinus may be composed of three pseudocryptic species from five geographical regions: (i) The true D. velutinus from India; (ii) D. sp. nov. 1 from Thailand, Vietnam and China; and (iii) D. sp. nov. 2 from Cambodia. While the morphology showed only subtle differences, the genetic divergences were remarkable, ranging from 6.8% to 8.2%. The Indian specimens were regarded as the true D. velutinus in the present study, but this needs to be confirmed by examining the currently unavailable type specimen. In addition, we propose that the two species, D. mineti and D. cylindricus, to be additionally incorporated into this species group based on molecular and morphological evidences. Lastly, nomenclatural problem of synonymizing a species without examining the type specimen was recognized in D. bobi under D. cylindricus, but further study is needed.

Keywords:*Coleoptera, Dorcus velutinus species group, Lucanidae, Pseudocryptic species, Taxonomy*

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O101M10
Taxonomy

The oldest fossil Cleroidea and Cucujoidea

Jiri Kolibac
Moravian Museum, Czech Republic

The Early Cretaceous and Middle Jurassic deposits of northern China have recently yielded rich fossil records of beetles. In particular, finds from the volcanic deposits of the Jiulongshan formation (c. 165 Ma) are among the oldest known beetle fossils of the suborder Polyphaga. Some of them are extraordinarily well-preserved and provide interesting insight into the evolution of morphological characters. In cooperation with Prof. Huang D.-Y. of the Nanjing Institute of Geology and Palaeontology, China, a rich body of material is studied, consisting of the Mesozoic family Parandrexidae and several extinct and extant cucujoid and cleroid families. The fossils exhibit a large number of interesting details including, for example, the mouth parts, cranial morphology, thoracic segments, and male and female genitalia. Implications for character evolution and phylogeny in Cucujiformia may therefore be considered.

Keywords:*Coleoptera, Cucujiformia, Cucujoidea, Cleroidea, Mesozoic*

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O102M01

Taxonomy

O102

The complete mitochondrial genome of the Mediterranean cryptic species of the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) complex

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The whitefly Bemisia tabaci is a species complex containing at least 28 morphologically indistinguishable cryptic species. Among these cryptic species, only the mitochondrial genome of the New World has been fully sequenced. The Mediterranean (MED) cryptic species of the B. tabaci complex is an important agricultural pest causing significant yield loss to crop production. In this study, we sequenced the complete mitochondrial genome of MED. The genome is a circular molecule of 15,890 bp, encodes 37 genes including 13 protein coding genes (PCGs), two ribosomal RNAs (srRNA and lrRNA) and 22 transfer RNAs. In addition, it contains two non-coding A+T-rich regions which are rarely found in other metazoan mitochondrial genomes except the New World cryptic species of the B. tabaci complex. All the stop codons of protein coding genes are TAA or TAG, except for coxII that terminate with an incomplete stop codon. 22 tRNAs were found and most of the tRNAs could be folded as classic clover-leaf structures, except the tRNA-SerUCU, which lose the DHU arm. Comparison of the evolution rate of the mitochondrial genes between MED and New World showed that COII has the lowest evolutionary rates while nd4/nd4L the highest. The complete mitochondrial genome of MED will provide new information to resolving the phylogenetic relationship of the B. tabaci species complex as well as identifying ideal genetic markers for cryptic species identification.

Keywords:*mitochondrial genome,Bemisia tabaci*

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O102M02

Taxonomy

O102

Phylogenetic analysis of the plant bug subtribes Monaloniina and Odoniellina (Heteroptera: Miridae: Bryocorinae)

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The plant bug subtribes Monaloniina and Odoniellina (Hemiptera: Miridae: Dicyphini) have been considered as closely related plant bug taxa, comprising 41 genera (Schuh & Slater, 1995). They are found mostly in the Afrotropical, Oriental and Australian regions, as well as islands of the southwest Pacific. Some species are important pests of foodplants, such as cocoa, cotton and cashews. Representatives of these subtribes are morphologically diverse, varying dramatically in size, coloration, vestiture, and presence or absence of exaggerated cuticular outgrowths. No comprehensive study has been undertaken of these character systems and the classification is untested. We report on a novel reclassification of these taxa, based on the first comprehensive examination of included taxa, resulting in the exclusion of four genera from these suprageneric groups. We present the results of two phylogenetic analyses, based on morphological characters of the Monaloniina and Odoniellina. The first analysis for the tribe Dicyphini s.l. demonstrates that these subtribes together form a monophyletic group with high support. The genus Felisacus, which was previously included to the subtribe Monaloniina, is excluded from this subtribe, and its relationship was found to be more distant to the redefined monaloniines. The second phylogenetic analysis of 79 terminal taxa, showed that the Monaloniina s.l. and Odoniellina s.l. are polyphyletic.

Keywords:*Taxonomy, phylogeny, morphology, Miridae, Monaloniina, Odoniellina*

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O102M03

Taxonomy

O102

The fossil reduviids (Heteroptera) of Messel (Germany) and Green River (USA)

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Two worldwide well-known fossil sites are the UNESCO World Heritage Site Grube Messel located in Germany and the Green River Formation in the United States of America. Both sites are of similar age with ~ 47 Ma (Messel) and ~ 50 Ma (Green River). The Heteroptera are a very interesting insect group, which until now has been only poorly studied in both sites. More than 1,700 specimens of Heteroptera from Messel and 1,100 specimens of Green River are stored in the collections of Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt am Main (Germany) and the National Museum of Natural History Smithsonian Institution, Washington D.C. (USA). With more than 6,600 extant species, the Reduviidae are the second largest group within the Heteroptera. Investigations on the systematic relationships among the assassin bugs are still in progress; e. g., different authors classified the Reduviidae from 21 up to 32 subfamilies. Until now, more than 60 fossil specimens of assassin bugs from Messel were found and the extant groups Harpactorinae and Elasmodeminae can be identified. Among the Harpactorinae, some fossils can be determined as Harpactorini, Ectinoderini and Apiomerini. Since extant Ectinoderini are restricted to the area between Sri Lanka and New Guinea, the new fossils enlarge the known geographical range of this group considerably. About 25 reduviid specimens were detected in the Green River collection; in regard to reduviids, this site seems to have a lower biodiversity than Messel.

Keywords:*Fossil reduviidae, Messel, Green River, Eocene*

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O102M04
Taxonomy

Fossil lace bugs (Heteroptera: Tingidae) from the Eocene

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A characteristic feature of lace bugs (Tingidae) is the distinctive lacelike network of raised ridges, which is present on the hemelytra and on the pronotum of two of the three subgroups. These subgroups comprise the Cantacaderinae and the Tinginae, while the Vianaidinae have a rather smooth body surface. Both adults and nymphs of the Tingidae are phytophagous. The 260 tingid genera with more than 2100 extant species are widely distributed in the different regions of the world. Their size ranges from about 2 mm to more than 8 mm. The fossil record of lace bugs comprises about 40 species, with oldest representatives in the Cretaceous. Most of the fossil taxa are described from amber, and here mostly from Eocene Baltic amber. Nevertheless, very well preserved specimens are also found in sedimentary rocks. Several fossil genera and species of lace bugs from the Eocene Fossillagerstatte Grube Messel (Germany) have been described, and now a new analysis of old and new findings is given. An ongoing study of heteropterans from the Eocene Fossillagerstatte Green River (USA) illuminates the poor biodiversity of tingids preserved in this site.

Keywords:*Heteroptera*

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O102M05

Taxonomy

O102

Taxonomic revision of the family Cixiidae from Korea (Homoptera: Fulgoromorpha)

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This paper completes a taxonomic revision of the planthopper family Cixiidae from Korea. Eight genera are recognized in the Korean fauna: Andes Stal, 1866, Atretus Emeljanov, 2006, Reptalus Emeljanov, 1971, Pentastiridius Kirschbaum, 1868, Oecleopsis Emeljanov, 1971, Cixius Latreille, 1804, Macrocixius Matsumura, 1914, Kuvera Distant, 1906. Among them, two genera, Atretus and Macrocixius, are recorded for the first time in Korea. Thirty six species, fourteen of which are new to science and eleven are new to Korea, are recognized in the Korean fauna: Atretus trifurcus sp. nov., Pentastiridius unjangsanensis sp. nov., P. seoguensis sp. nov., P. jillyangensis sp. nov., Cixius jejudoensis sp. nov., C. mayisanensis sp. nov., C. obongsanensis sp. nov., C. pohyonsanensis sp. nov., C. sigmoidensis sp. nov., C. wanjonensis sp. nov., C. weonhyosanensis sp. nov., Kuvera hallasanensis sp. nov., K. hagilsanensis sp. nov., K. yecheonensis sp. nov., Reptalus iguchii (Matsumura, 1914), P. hachijonis (Matsumura, 1914), P. harimaensis (Matsumura, 1914), C. flavescens Matsumura, 1914, C. hakonensis Matsumura, 1914, C. heydenii Kirschbaum, 1868, C. ibukisanus Matsumura, 1914, C. nawae Matsumura, 1914, C. towadensis Matsumura, 1914, C. okinawanus Matsumura, 1936 and Macrocixius giganteus Matsumura, 1914. New combinations proposed are: Reptalus iguchii (Matsumura, 1914) comb. nov., Pentastiridius hachijonis (Matsumura, 1914) comb. nov. and P. harimaensis (Matsumura, 1914) comb. nov. Previously recorded three species from Korea are not available in current investigation: Cixius nervosus (Linnaeus, 1758); Kuvera ligustri Matsumura, 1914 and Pentastiridius angusticeps (Horvath, 1892). Identification keys to tribe, genera and species are provided.

Keywords:*Planthopper, Fulgoroidea, Cixiidae, Andini, Pentastirini, Cixiini, Korea*

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O102M06

Taxonomy

O102

Systematic review of the sub-family Phylinae (Heteroptera: Miridae) in the Korean Peninsula with discussion on phylogenetic relationship within family Miridae

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The Korean Peninsula is located in temperate ecozones which shelter Palearctic flora and fauna. Because of small size and easily fragileness the tiny phyline bugs has been poorly studied from this region. Herein, this study tries to accomplish revision of subfamily Phylinae (Miridae) in the Korean Peninsula which document a total of 85 species in 26 genera. Due to the survey from 2008 to 2011 many species are described and reported which were fragmentally reported (Duwal and Lee, 2011, Duwal, et al., 2010; 2012 (to be published)) and many species await description. Many species like Campylomma chinense Schuh, 1984; C. miyamotoi Yasunaga, 2001; Europiella artemisiae (Becker, 1864); E. kiritshenkoi Kulik, 1975; E. miyamotoi (Kerzhner, 1988), Pherolepis kiritshenkoi (Kerzhner, 1970) are reported for the first time in Korea. Tytthus koreanus Josifov and Kerzhner, 1972 and Hallodapus fenestratus Linnavuori, 1961 are synonymized to Tytthus chinensis (Stal, 1859) and Hallodapus centrimaculatus (Poppius, 1914) respectively, and Europiella lividella (Kerzhner, 1973) and Parapsallus vitellinus (Scholtz, 1847) are re-combined to their original status. The feeding habits of phyline bugs are not clear though most of them are considered as phytophagous, but tribes like Hallodapini, Pilophorini and some groups of phylines are also considered as predatory/ or omnivorous. Finally, phylogenetic analysis of Family Miridae will be discussed including morphometric and molecular data to see the relationship and placements of groups within Subfamily and tribes.

Keywords:*Hemiptera, Heteroptera, Miridae, Phylinae, Revision, the Korean Peninsula, Phylogeny Miridae*

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O102M07

Taxonomy

O102

Faunistic studies of tribe Halyini (Hemiptera, Pentatomidae, Pentatominae) of Pakstan with description of nine new species

Abdul Manan Shaikh[1], Nasreen Memon[2], Manssor Ali Shah[3], Nadir Ali Birmani[4]

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According to available literature a large number of species have been added to the tribe Halyini from time to time and also place of different species changed from one genus to other by different workers, new records are also explored time to time, because of this, their distributional range further increased. There are about 31 genera and more than 110 species from Pakistan and different areas of adjoining countries are described by different authors. During present study collection of Berry Bugs species were carried out from different areas of Pakistan. A total of 5600 specimens were collected and preserved as per standard procedure. The material was studied, compared and classified according to available literature. During present studies, twenty six species are studied including nine new species of four genera are described. In addition seventeen already described species of seven genera have also been collected and studied from different localities and different host plants in Pakistan.

Keywords:*Hemiptera, Halyini, Pakistan, new species*

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O102M08

Taxonomy

O102

Revision of Asian species of genus *Halys* Fabricius (Hemiptera: Pentatomidae: Halyini) in addition to description of new species from Pakistan

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University of Sindh, Pakistan

Halys is a cosmopolitan oriental genus of tribe halyini, found in almost all parts of the world. In Pakistan they are found on a number of food plants and plants of commercial importance such as Shesham, Babur and Mulberry trees. Present study includes eight species from oriental region of Asia; H. fabricii Fabricius, H. nilgiriensis Distan, H. shaista Ghauri, H. serrigera Westwood and H. sulcatus, including three new species H. hyderabadiensis n. sp, H. mulbariensis n.sp, H. noakotensis n.sp, one from Punjab province and two from Sindh provinces, Pakistan respectively. The new species are described on the basis of external morphological characters and the description of internal and external male and female genitalia with special references to scent gland apparatus. The new species are compared with their closely allied species, and key of eight species including new species is given here. The localities of new species are addition in the geographical distribution of genus halys in the existing literature.

Keywords:*Halyini, Asia, halys, new species, Pakistan*

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O103M01
Taxonomy

O103

Three new species of the genus *Polypedilum* Kieffer (Diptera: Chironomidae) from the Yaeyama Islands, Japan, with comments on the taxonomic implication of the superior volsella

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[1]Osaka Prefecture University, Japan, [2]Shimonoseki, Yamaguchi, Japan, [3]Osaka Prefecture University, Japan

Polypedilum Kieffer, 1912 is one of the largest genera in the family Chironomidae and at least 115 species have been described in Japan. According to Sæther et al. (2010), this genus has been divided into eight subgenera and the structure of the superior volsella is treated as one of the most important taxonomic characters for separating the subgenera and inferring the phylogenetic relationship among them. In the present study, three new species from the Yaeyama Islands, the Ryukyus, Japan, are described and figured based on male adults. Two of the new species, Polypedilum sp. 1, and P. sp. 2, are assigned to the subgenus Polypedilum. One species, Polypedilum sp. 3, is not assigned to any subgenus due to the unique structure of the superior volsella, and it may be justified to erect a new subgenus for it in future study. We would in any case hesitate to erect a new subgenus based solely on it, because we are rather sceptical whether the structure or shape of the superior volsella should be treated as one of the most important taxonomic characters for separating the subgenera. This species contains elements of both Uresipedilum and a part of Tripodura on the shape of superior volsella. Furthermore, as the shapes of the superior volsellae are highly variable, it appears that a tendency of continual variation is present throughout each subgenus. These might destabilize some positions of the subgenera defined by the features of the superior volsella in the genus Polypedilum.

Keywords:*Chironomidae, Poypedilum, three new species, superior volsella, Yaeyama Islands, Japan*

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O103M02

Taxonomy

O103

India bluetongue vector network: Molecular and morphological taxonomy of south Indian *Culicoides*

Lara Ellen Harrup[1], Israel Stalin[2], Y Krishnamohan Reddy[3], Susmitha Sudheer[4], Y Narsimha Reddy[5], S M Byregowda[6], Basanti Brar[7], Minakshi Prasad[8], Gaya Prasad[9], Bethan Purse[10], Mudassar Chanda[11], David Rogers[12], James Logan[13], Clare Brand[14], Mike Birkett[15], John Pickett[16], Simon Carpenter[17]

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Bluetongue (BT) is an infectious, non-contagious disease of ruminants which inflicts major losses on subsistence sheep farmers in southern India. The agent that causes this disease (bluetongue virus: BTV) is transmitted by Culicoides biting midges (Diptera: Ceratopogonidae). The India Bluetongue Vector Network (IBVNet) is a three year Indo-UK collaborative project aiming to examine ways to both predict and reduce the impact of BTV by monitoring monsoon-related Culicoides activity and exploring affordable methods for their control. Key to developing multi-state surveys of Culicoides populations, and vector competence and control studies is a sound taxonomic understanding of the livestock-associated Culicoides present. To achieve this livestock-associated adult Culicoides were collected using ultraviolet (UV) light-suction traps from three states in southern India severely affected by BTV outbreaks (Andra Pradesh, Karnataka and Tamil Nadu). Using a combination of non-destructive DNA extraction, DNA barcoding and preservation of slide-mounted voucher specimens, high quality digital images of key morphological characteristics were made and assessed in relation to the results of phylogenetic analysis of the corresponding DNA Barcode sequences produced for the mitochondrially encoded cytochrome c oxidase I (MT-CO1) region. The results of this analysis are being used to generate a taxonomic guide to the Culicoides of southern India relevant to BTV epidemiology and place the Indian Culicoides fauna in a global perspective.

Keywords:*Culicoides, Ceratopogonidae, Bluetongue Virus, India, DNA Barcoding*

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O103M03

Taxonomy

O103

A systematic study of the genus Chrysotoxum Meigen (Diptera, Syrphidae) in Korea

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The family Syrphidae is distributed worldwide, with the greatest species diversity in the New World Tropics. More than 6,000 species have been described so far. The genus Chrysotoxum Meigen includes fairly large flies with body coloration similar to wasps. There are over 100 known Chrysotoxum species of which 74 species occur in the Palaearctic region. They can be easily distinguished from other genera by the follow characteristics: 1) humeri bare; 2) antenna usually longer than head and positioned horizontally in lateral view; 3) wing vein R4+5 dipped posteriorly; and 4) abdomen black with yellow bars or bands or characteristic black arcuate markings. As a result of systematic study of Korean Chrysotoxum, we have recognized 10 valid species, including two species new to science, two newly recorded species in Korea (C. tuberculatum, C. ramphostomus), and six previously known Korean species (C. biguttatum, C. coreanum, C. grande, C. sapporense, C. sibiricum, C. vernale). In addition, we synonymized C. shirakii with C. sapporense. We here provide a key and photographs with their key characters indicated.

Keywords:*Diptera, Syrphidae, Chrysotoxum*

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O103M04
Taxonomy

O103

Ulidiidae (Diptera) systematic - Molecular and morphological aspects

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Ulidiidae is a small Tephritoidea family. Ulidiidae are often combined with Otitidae in one family, named Ulidiidae or Otitidae. Our analysis of the genital structures revealed that Ulidiidae + Otitidae form a paraphyletic group, referring to derived Tephritoidea. Therefore, we consider Ulidiidae and Otitidae as separate families. We studied sequences of the COI gene of 58 specimens of 25 species of Ulidiidae and Otitidae. COI was shown to be an effective marker to distinguish Ulidiidae and Otitidae species, except for the genus Melieria (Otitidae). Melieria shows the highest level of intraspecies differences and smallest - of interspecific with high support at all cladograms. This suggests that Melieria is a young genus, being is at the stage of intense speciation. Monophyly of the family Ulidiidae with the inclusion of Homalocephala not confirmed on any of the molecular reconstruction. The position of the genus Homalocephala is indefinite. On the MP and NJ cladograms it forms a sister group to the Otitidae. On the ML and Bayes cladograms Homalocephala species are included in clusters of both families as their basal elements. Our study of genital structures has shown that the genus Homalocephala cannot be included neither in Ulidiidae, nor in Otitidae. It represents the separate clade within the Tephritoidea, which requires further study. In all cladograms genera Timia and Ulidia form mixed cluster. This is consistent with the morphological data on which these genera are very close to each other under the external morphology and genital structures and have little or no hiatus.

Keywords:*Ulidiidae, Diptera, systematic, phylogeny, barcoding*

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O103M05

Taxonomy

O103

Systematic study of the family Bombyliidae (Diptera: Asiloidea) in Korea

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Bee flies (Diptera: Bombyliidae) are distributed worldwide with more than 4,500 known species. This family occurs in broad variety of habitats and ecosystems, but its highest species diversity concentrated in arid and semi-arid regions. Adult bombyliids mostly feed on nectar of flowers, and immatures are poorly known. According to the previous records, they are primarily parasitods of other insects. In the process of revising Korean Bombyliidae, we have recognized 31 species of 10 genera, among which five species are new to Korea. Unfortunately, we still have not confirmed 7 previously recorded Korean species. Among them, we believe that we have not collected Bombylius lejostomus (TL: North Korea) and Ligyra flavofasciata (TL: China) yet, and other five species could possibly be based on erroneous previous identifications. In addition, we recognized nine species identified only to generic level. They are possibly either new species or uncertain species needed more investigation for accurate identification. We here provide an updated list of Korean Bombyliidae, a key, and photographs with their key characters indicated.

Keywords:*Bombyliidae, Bee flies, list of Korean Bombyliidae, key, photographs*

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O103M06

Taxonomy

O103

Utility of COI, COII and ND4 genes for discriminating forensically important Diptera

Alicia Eva Timm[1], Trevor Stamper[2], Gregory Dahlem[3], Ronald Debry[4]

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One of the most debated topics in molecular taxonomy concerns the region of DNA that is used to identify species. For forensic entomology, where species are well characterised, it is worth exploring whether or not some DNA region that is different from the standardized barcode region can provide better species discrimination. Our objective was to investigate the use of COI and COII, the two most commonly used genes for identifying species of this group, as well as the ND4 gene, which shows promise for robust species identification. Since the use of molecular identification is most challenging among closely related species sister species, pairs of different genera of the Calliphoridae and Sarcophagidae were included in analyses. The data set was analysed using the three concatenated genes, each individual gene, and sliding windows consisting of 600bp. Of the three genes analysed, COI yielded the highest level of total intraspecific nucleotide diversity, followed by COII and ND4. Within the COI gene, the optimal location for species discrimination was situated towards the 3' end of the gene. The effect of fragment length on nucleotide divergence and phylogeny was investigated by examining fragments with 200bp increments. Results showed that fragment lengths of at least 1000bp are necessary to ensure that close to 100% total diversity is sampled. Therefore, we recommend that the optimal fragment for identifying forensically important Diptera is located towards the 3' end of the COI gene and consists of 1000bp to ensure greater diversity coverage.

Keywords:*Diptera, molecular taxonomy, cytochrome oxidase, ND4*

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O103M07

Taxonomy

O103

A systematic revision of the Korean Lauxaniidae (Insecta: Diptera)

Hyun-Suk Lee[1], Ho-Yeon Han[2]

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The Lauxaniidae is one of the large families of acalyptrate flies (about 1,550 world species under 142 genera). They are known to occur in all the zoogeographical regions. Adult flies are found in a wide range of habitats. They are common in moist shady undergrowth, grassland, all forest types. Their larvae are saprophagous and are found in leaf litter, decaying vegetation and similar habitats. The family can be distinguished from the other acalyptrate flies by the combination of the following characteristics: 1) postocellar seta inclinate to cruciate, 2) true vibrissae absent, 3) dorsal preapical seta present on tibiae and 4) costal vein not broken. In Korea, the number of previously recorded species is 33, among which 10 species have been given Korean names. As a result of our systematic investigation of the Korean Lauxaniidae, we have recognized 95 species, including 34 newly recorded Korean species, 21 possible new species and 7 species with uncertain identity. We conducted a cladistic analysis to infer generic relationships among the genera in Palaearctic region. We also inferred specific relationships of six selected genera in Korea. We also made DNA barcoding analysis, which turned out to be highly effective in resolving some taxonomic problem caused by sibling species complex.

Keywords:*Diptera, Lauxaniidae, Taxonomy, DNA barcoding, Cladistics*

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O103M08

Taxonomy

O103

Heterogeneity and prevalence of cryptic species in *Wolbachia* infected uzifly *Exorista sorbillans* (Diptera:Tachinidae)

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Inherited bacterial symbionts from the genus Wolbachia have attracted much attention by virtue of their ability to manipulate the reproduction of their arthropod hosts. Speciation in insects induced or facilitated by symbionts Wolbachia which can manipulate host reproduction and induce reproductive isolation. The potential importance of these bacteria has been underlined by surveys, which have estimated that 20% of insect species are infected. Cytoplasmic Incompatibility (CI) is the one of the important reproductive manipulation induced by the Wolbachia. Previous studies have clearly established high prevalence of Wolbachia infection in the Uzifly Exorista sorbillans a tachinid endoparasite of silkworm. In the present investigation we detected the heterogeneity and cryptic species in E. sorbillans infected with Wolbachia. The DNA finger printing of infected populations was done by using the Randomly Amplified Polymorphic DNA (RAPD). The dendrogram constructed from Un-weighted Pair Group method with Arithmetic Mean (UPGMA) and distance computed using Elucidation distance clearly detected the heterogeneity and prevalence of cryptic species in E. sorbillans populations. This could be the result of geographic isolation and Cytoplasmic Incompatibility (CI) induced by Wolbachia among the Uzifly field populations.

Keywords:*Wolbachia, CI, speciation, Exorista sorbillans*

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O103M09

Taxonomy

O103

Phylogeny of the mosquito tribe Culicini (Diptera, Culicidae) based on COI sequences and morphology.

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The tribe Culicini comprises four genera, including Culex which includes 768 species classified in 26 subgenera. Several of these species are vectors of arboviruses (e.g. West Nile and Japanese encephalitis viruses) and filarial worms that cause disease in humans. Within the framework of an integrated morphological and molecular systematics project, we carried out the first fully integrated study on the phylogenetic relationships within this tribe to investigate and understand the evolution and diversification of this group. Selected species of the tribe Culicini representing all the different genera, subgenera and species groups were sequenced for the COI barcode region and analyzed. We discuss their variability and the phylogenetic signal held separately by the DNA sequences and morphology, and by the combined data, with the aim at elucidating the phylogenetic relationships of supraspecific taxa of this species-rich group.

Keywords:*Culicini, phylogeny, COI, morphology*

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O104TU01

Taxonomy

O104

The phylogeny of chelonine wasps (Hymenoptera: Braconidae) with special reference to the species diversity of the Australian fauna

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The Cheloninae are a diverse subfamily of braconid wasps which have a global distribution. There are about 1,300 described species in 15 genera, but only 43 species (incl. five introduced species) in four genera are recorded for Australia. Chelonines are egg/larval parasitoids of Lepidoptera and they can be easily distinguished from most other braconids by the presence of a metasomal carapace, where the first three tergites are fused into a dorsal plate. This study aims to assess the phylogenetic relationships among world genera employing sequence data from three genes, cytochrome c oxidase I (CO1), 28S (rDNA) and ef1α (nuclear DNA). Preliminary results show that most genera are monophyletic with the exception of Microchelonus (characterised by the presence of a large pit on the posterior metasoma), which falls within Chelonus. Genera belonging to the tribes Pseudophanerotomini and Phanerotomini, in which all species have sutures on the carapace, form a natural group but neither of the tribes is monophyletic. The species diversity of the Australian fauna, assessed using an integrated approach of COI data and morphology, is huge. The four common genera total more than 70 species, with new taxa found in all major habitat types including the arid zone of central Australia as well as coastal mesic environments.

Keywords:*CO1*

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O104TU02

Taxonomy

O104

Phylogeny and higher classification of Mutillidae (Hymenoptera): Re-evaluation based on morphology

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In 1975 Brothers produced the first phylogenetic analysis and higher classification of Mutillidae (velvet ants) on a world-wide basis, using 43 characters and groundplans for the taxa. Seven subfamilies and several tribes and subtribes were proposed. In 1997 Lelej & Nemkov published an analysis (using 89 characters, many different from those previously used at this level) and proposed ten subfamilies, reflecting several differences in inferred relationships. Slight expansion and reanalysis by Brothers in 1999, taking Lelej & Nemkov's results into account, produced essentially the same results as he had obtained in 1975, however. More than 50 genera, including varied species for each where possible, were individually scored for all morphological characters previously considered in such analyses as well as others used at the genus level, a total of about 190 characters. Four outgroup genera representing those families previously found to be most closely related to Mutillidae were similarly scored. Parsimony analyses were performed, using various options involving additivity of character states and implied weighting of characters. Neither Brothers's nor Lelej's previously proposed phylogenies were fully supported. Several 'taxa' were found probably to be paraphyletic. Different analytic assumptions produced considerable differences in branching patterns. Recognition of a series of higher taxa somewhat intermediate between the earlier schemes proposed by Brothers and Lelej may be justified. However, the inclusion of several characters polymorphic within genera, and the fact that such variation in state expression appears to differ in extent in different parts of the trees found, has undoubtedly confounded the situation.

Keywords:*Hymenoptera, Mutillidae, phylogeny, higher classification*

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O104TU03

Taxonomy

O104

The phylogeny of Tenthredinidae (Hymenoptera): A morphological perspective

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The Tenthredinidae with more than 5.000 described species is the largest family of non-apocritan wasps. The members of this and the other families in the Tenthredinoidea are all herbivorous and are commonly known as sawflies. Tenthredinidae has no obvious autapomorphies and has not been retrieved as monophyletic in any stringent phylogenetic analyses. On the contrary, most other families (Argidae, Cimbicidae, Diprionidae, and Pergidae) in the Tenthredinoidea have repeatedly been placed inside the Tenthredinidae. The current subfamily classification has never been tested cladistically. To evaluate the status of the Tenthredinidae and its subdivisions, a comprehensive morphological data set of 150 characters has been scored for 130 taxa. Character systems are all derived from adult anatomy and include the head, thorax, wings and other body appendages, and the ovipositor apparatus. The data set was analysed with parsimony software under various settings. Preliminary results confirm the monophyly of Tenthredinoidea, the Blasticotomidae being the sister group to all remaining Tenthredinoidea, and the monophyly of Cimbicidae, Diprionidae, and Argidae + Pergidae. The Tenthredinidae is paraphyletic, and none of the subfamilies currently recognized are retrieved as monophyletic under all analytical settings. Topologies are not stable, and many nodes are weakly supported and liable to disappear when the analytical settings are changed. To obtain a robust phylogeny that may serve as the basis for a reclassification and interpretation of the evolutionary history of Tenthredinidae/Tenthredinoidea, it is necessary to tap additional sources of information, first and foremost molecular.

Keywords:*sawflies, 'Symphyta', classification, evolutionary history, herbivorous insects*

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O104TU04

Taxonomy

O104

Taxonomic confusion and misidentification of multiple invasive ant species - Multiple current species are species complexes.

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Here we detail confusion surrounding the taxonomy and identification of two apparently invasive ant species, Iridomyrmex anceps Roger and Ochetellus glaber Mayr, which are commonly cited in the literature as having been spread widely by human commerce. We provide morphometric data and images strongly suggesting that these two "species" are instead complexes of species that are likely native to many regions where they are purportedly introduced. We take particular interest in the status of species referred to as I. anceps and O. glaber in China, as this is the most northerly distribution of both genera. We then expand our analyses to the recent morphologically based revision of the genus Iridomyrmex (Heterick and Shattuck 2011) as we believe that this revision unjustifiably combines taxa that are very obviously multiple and in several cases very many species, with clear morphological differentiation between populations that are often sympatric and having up to 12% genetic divergence based on CO1 analysis.

Keywords:*taxonomy, ant, Iridomyrmex, Ochetellus*

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O104TU05

Taxonomy

O104

Filling the gaps - New lower Cretaceous Hymenoptera and the early evolution of the order

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The early evolution of the megadiverse insect order Hymenoptera is of general biological interest as it was accompanied by a dramatic change in biology from phyto- and xylophagy (i.e. sawflies and woodwasps) to parasitoidism (Euhymenoptera, i.e. Orussidae + Apocrita). The phylogeny of Hymenoptera is briefly reviewed based on recently published morphological and molecular data sets, and then used as a framework to examine the biological and phylogenetic implications of two recently discovered fossils from the Lower Cretaceous Crato Formation of Brazil. A new fossil sawfly species is placed within Tenthredinoidea and characterized by distinctly biflabellate antennae. This morphological feature indicates that long distance mate finding behaviour (as exhibited by males of recent Diprionidae and some Pergidae and Tenthredinidae) was already well established in the Lower Cretaceous. The phylogenetic position of a second fossil, the woodwasp Cratoenigma articulata gen. et. sp.n., is discussed on the basis of relevant thoracic, abdominal and wing venation characters. The latter fossil cannot be placed in any existing superfamily but based on a combination of plesiomorphic, synapomorphic and autapomorphic character states it forms the putative sister group of Xiphydriidae + Euhymenoptera. This would place it well within Unicalcarida, the clade in which the transition from endophytic to a parasitoid lifestyle evolved. These two fossils close significant gaps in the fossil history of Hymenoptera and provide new insights into the Cretaceous diversity of the order.

Keywords:*Hymenoptera, systematics, evolution, phylogeny*

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O104TU06

Taxonomy

O104

Why was the genus *Amblyopone* split from the genus *Stigmatomma?* (Hymenoptera: Formicidae) - Mandibular evolution in Dracula ants told from males -

Masashi Yoshimura

California Academy of Sciences, U.S.A.

Ants have a distinct sexual dimorphism. The morphological characters observed in male ants could provide useful hints to determine homologies among strongly specialized characters in worker ants. The workers of Amblyopone and Stigmatomma in the subfamily Amblyoponinae, which are known as "Dracula ants," have a specialized elongate mandible with serriform. On the other hand, the male mandibles in both genera are small and simple. A comparative study of the mandibles among males and workers, and the two genera was carried out to better distinguish Amblyopone and Stigmatomma. The results of this study revealed that the number of mandibular teeth in males is different between Amblyopone and Stigmatomma, and that the difference in mandibular characters reflects the different scenarios for the evolution of the worker mandible in each genus. The difference in the two scenarios is associated with the original open-close position before the mandibles evolved to be longer. Furthermore, Stigmatomma shares the same mandible characters with the other members of the XMAS clade (Xymmer + Mystrium + Adetomyrma + Stigmatomma), which is strongly supported by a recent molecular phylogenetic study (Brady et al. 2006), while Amblyopone does not. These results provide robust evidence that the genus Amblyopone resides outside of the XMAS clade.

Keywords:*phylogeny, Male-based revision, Malagasy region, morphology, Amblyoponinae, Amblyoponini, Adetomyrma*

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O104TU07

Taxonomy

O104

Employing polydnavirus genes to uncover cryptic species in Ichneumonoidea

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Parasitoid wasps are major components of habitat biodiversity and are important in maintaining ecosystem stability. They can also offer an effective, highly specific and environmentally friendly alternative to chemical insecticides. Their success as biocontrol agents depends entirely on our understanding of their biology and phylogenetic relationships -examples of failure where parasitoid wasps have been integrated into a pest management program are all too often the result of incorrect species identification and inadequate knowledge of population genetics. Additionally, correct identification of parasitoid wasps is essential if we are to make accurate estimates of biodiversity in order to locate biodiversity 'hotspots' on which conservation efforts can be focused. I address these issues by examining fast-evolving polydnavirus genes within wasp nuclear DNA. These genes are thought to function to attack the host immune system, and if their variability correlates with host preference they could aid in the identification of cryptic 'specialists' within COI-barcoded 'generalist' species. Correct identification of parasitoid wasps using polydnavirus polymorphism as an indicator of host preference could vastly improve conservation efforts, the success of biocontrol programs and our general understanding of food-web ecology.

Keywords:*sibling species, species richness, biodiversity, parasitoid, Ichneumonidae, ichnovirus*

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O104TU08

Taxonomy

O104

Morphology and molecules for the masses: remarkable species diversity in a large and taxonomically challenging genus of ants.

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Monomorium Mayr (Hymenoptera: Formicidae) is a highly abundant, widespread and diverse genus of ants. Several Australian "species" show considerable morphological and chemical variation, while evidence from ecological and preliminary molecular research suggests significant cryptic diversity. However, rigorous morphological criteria that support separate species have proved difficult. In this study we are using sequence data from multiple loci to generate a phylogeny for the Australian species in order to test the current species group relationships. In addition, we generate hypotheses for species boundaries in the widespread and morphologically diverse seed harvesting M. "rothsteini" complex using a combination of molecular, morphological and distributional data. Preliminary results indicate that the Australian Monomorium species groups, as currently understood, are not supported by the molecular phylogeny and that several groups may be para- or polyphyletic. Our results also provide strong evidence for a large number of unrecognised species in the M. "rothsteini' complex and other morphologically diverse groups and demonstrate the importance of using multiple data sources when making taxonomic decisions.

Keywords:*Monomorium, species boundaries, molecular phylogeny, mtDNA lineages*

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O104TU09

Taxonomy

O104

New insight into an old genus: Revising *Disholcaspis* Dalle Torre & Kieffer, 1910 (Hymenoptera: Cynipidae)

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As with many Nearctic cynipid genera, the genus Disholcaspis is in desperate need of an updated circumscription. This project seeks to combine a systematic revision of Disholcaspis with an exploration into patterns of host plant usage. Disholcaspis contains over 50 valid species all of which are known to utilize white oaks (Fagaceae, section Quercus) as host plants. Recently described species (from Costa Rica and Panama) have expanded the known distribution of Disholcaspis to a range reaching from Canada to Panama. Many species of cynipids are heterogenetic, with alternating sexual and asexual generations that are not only morphologically distinct but also produce galls on different parts of the same host plant. Disholcaspis wasps are generally only known from their asexually reproducing generations, many of which induce woody stem galls that are often morphologically species specific. The sexual generations within the genus remain mostly unknown, with only those of Disholcaspis quercusvirens (Ashmead) and Disholcaspis cinerosa (Bassett) having been identified, and those of Disholcaspis eldoradensis (Beutenmuller) and Disholcaspis quercusmamma (Walsh & Riley) formally described. The sexual generations have been found to develop within buds and their galls have been found to be morphologically similar across species. Analysis of morphological and molecular data from both generations will aid in clarifying the classification within Disholcaspis as well as give insight into the evolution of plant-insect interactions.

Keywords:*Cynipidae, Disholcaspis*

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O104TU10
Taxonomy

O104

Phylogeny of Dryinidae: Evolution of Fulgoromorpha parasitism

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Tokyo University of Agriculture, Japan

The family Dryinidae comprises parasitoid wasps of Auchenorrhyncha (Homoptera). Females in most subfamilies of Dryinidae have raptorial forelegs that aid in grasping the host insect. The host range is different depending on the taxon. The taxa bearing less developed foreleg parasitize Cicadomorpha, and the taxa bearing more developed foreleg parasitize Fulgoromorpha. Although most members are Fulgoromorpha parasitoids, Gonatopodinae include Cicadelidae parasitoids which have comparatively less developed foreleg, and Bocchinae also parasitize both, but the chela of Bocchus females which parasitize fulgoromorphan family Issidae is quite different from other dryinid taxa. Olmi (1994) explained the evolution of the raptorial foreleg in the view of the activity of host insects, however, the phylogenetic relationship of dryinid taxa has been unclear (Carpenter, 1999). As a result of the cladistic analyses based on 53 morphological characters from 36 species (10 genera, 6 subfamilies), the subfamilies including Fulgoromorpha parasitoids, i.e., Bocchinae, Dryininae and Gonatopodinae clustered single clade. On the other hand, the subfamilies of Cicadomorpha specialists were paraphyletic. Although further study is needed to test the phylogenetic position of Bocchinae, the results supported the synchronous development of foreleg and Fulgoromorpha parasitism. The treatment of some Cicadelidae parasitoids of Gonatopus is still uncertain, but the Fulgoromorpha parasitism is considered as monophyletic. At least (Dryininae + Gonatopodinae) linage can be defined by 6 autapomorphies, and 4 of them are about foreleg morphology. It may explain that the developed foreleg is required because gripping fulgoromorphan insect is more difficult than Cicadomorpha.

Keywords:*Hymenoptera, Chrysidoidea, parasitoid, host information*

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O104TU11

Taxonomy

O104

Comparative mitochondrial genomics of Hymenoptera

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Animal mitochondrial genes and genomes have been widely used in population genetics, species identification, phylogenetic studies and evolution. Recent years, many studies focused on the comparative and phylogenetic studies of insect mitochondrial genomes. Hymenoptera, as one of the most species richness and diverged group, their mitochondrial genomes show unique features compared with those of other insects. Presently, 32 mitochondrial genomes have been sequenced from Hymenoptera, representing 15 families. There are five characteristics in the mitochondrial genomes of Hymenoptera: (1) high A+T content in most species of Apocrita; (2) reversal of strand asymmetry in the Family of Braconidae; (3) extremely fast on the evolutionary rate compared with nuclear genes in some lineages; (4) frequent and evolutionary independent rearrangement of tRNA gens; (5) accelerated protein-coding gene rearrangement in some lineages. The phylogenetic study using complete mitochondrial genomes sequence indicate a basal position of Hymenoptera in holometabolous insect and novel phylogenetic relationships with this order.

Keywords:*mitochondrial genomes, gene rearrangement, evolutionary rate*

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O104TU12

Taxonomy

O104

A molecular phylogeny of Bethylidae (Hymenoptera: Chrysidoidea) based on mitochondrial and nuclear genes

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A molecular phylogeny of the family Bethylidae (Hymenoptera: Chrysidoidea) was conducted based on a total of 49 species consisting 43 ingroup species of 19 genera and 6 outgroup species by using 4 molecular markers, mitochondrial 16S rRNA, CytB, and nuclear 18S rRNA, 28S rRNA genes. Data were analysed using maximum likelihood (ML), parsimony and Bayesian inference (BI) phylogenetic frameworks. As results, followings are confirmed: (1) sister group relationships of Chrysididae and Bethylidae; (2) monophyly of Bethylidae; (3) monophyly of Bethylinae, sistered to the rest of Bethylidae; (4) monophyly of Epyrinae is not supported, as Epyrinae is divided into two separated clades, Epyrini and Sclerodermini; these tribes sistered to subfamilies, Pristocerinae and Mesitiinae, respectively; (5) monophylies of the other subfamilies, (e.g. Pristocerinae). These results are largely congruent with former hypotheses based on morphological and molecular data with respect to the monophyly of various subfamilies and tribes. Some phylogenetic relationships are also found at lower level: (1) a complex clade consisting of three genera, Acrepyris, Apenesia and Pristocera; (2) Laelius and Formosiepyris is more close to Holepyris than Epyris; (3) Sclerodermini of Epyrinae is the sister group of a clade of Pristocerinae+Epyrini. Based on these results, we discussed on the phylogenetic relationships of Bethylidae comparing to morphological and traditional classifications.

Keywords:*Bethylidae, Chrysidoidea, molecular phylogeny, mitochondrial gene, nuclear gene*

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O104TU13

Taxonomy

O104

Morphometric and landmark based variations of *Apis mellifera* L. wings in the forest vegetation zone of Nigeria

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212words There are several different methods of classifying insect. The technique of using wing landmarks and morphometric features to discriminate insect population into species and specify the variations in the varieties within the intra specific group was used to classify Apis mellifera kept in beekeeping practice in the forest vegetation zone of Nigeria into morphoclusters. The multivariate morphometric data obtained was analysed with parametric statistic tools of mean, standard deviation and standard error. The distribution and relation between them were subjected to two step cluster analysis. Morphoclusters means were presented in centroids and also the simultaneous confidence intervals (95%) of means values of wing morphometric and landmarks were expressed. Varieties of Apis mellifera found in this study formed four distinct morphoclusters based on wing landmarks and morphometric. Osun State recorded morphoclusters 1 and 2 while Ebonyi and Oyo States had morphoclusters 3 and 4 respectively. 20 landmarks that occurred on the forewing of morphoclusters 1, 3 and 4 were the same while the record of 19 landmarks observed in honeybees of morphoclusters 2 was different from the existing records. Based on our findings in this study it is reliable to use the variations on the wing landmarks and morphometric of honeybee workers to group Apis mellifera in Nigeria into varieties of existing species.

Keywords:*Apis mellifera, Wing landmarks, Morphometric features, Cluster analysis.*

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O104TU14

Taxonomy

O104

Survey on biology and distribution of *Biorhiza pallida* Olivier (Hymenoptera: Cynipidae) and identification of its parasitoids in West-Azerbaijan, Iran

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The species named oak apple gall wasp belongs to family Cynipidae and tribe Cynipini. From 2009 to 2010, field samplings of produced galls on Quercus infectoria Olive. were conducted and collected galls were identified with recordings of date and the location of collection. The galls were transferred into plastic container dishes in order to check emergence and for identification of natural enemies, especially parasitoids. The gall produced by Biorhiza pallida was rounded and looked like apple fruit. The B. pallida galls were bright-green with bright-red surface and were produced on the branches of Q. infectoria. This gall was multichambered and was produced by sexual form of this species in the spring (May and June). The first galls emerged in mid-May and maximum growth of galls occurred at the end of June and at that time diameter of galls was 10?20 mm. Asexual form of B. pallida produced the galls that looked like potato on root of Q. infectoria in the summer. Distributions of the produced gall by B. pallida were in locations such as Ghabre-hossein, Banokhalaf, Zamziran, Rabat and Mirabad areas. The most important point about these regions was that their climates were the same. The identified parasitoids of B. pallida were: Torymus erucarum, Torymus flavipes, Cecidostiba fungosa, Sycophila biguttata, Sycophila variegate, Ormyrus pomaceus, and Mesopolobus sp.

Keywords:*Gall, Cynipidae, Parasitoids, Forest*

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O105TU01

Taxonomy

O105

Phylogenetic relationships of Agaristinae (Lepidoptera: Noctuidae) based on adult morphology, with special reference to the biogeography of "sound-producing clades" and the evolution of diurnality

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[1]National Taiwan University, Taiwan, [2]National Taiwan University, Taiwan, [3]National Sun Yat-sen University, Taiwan

The Agaristinae represents a large subfamily of Noctuidae, consisting of 127 genera and about 500 species ranging throughout all the major continents except Antarctica and Greenland. The adults of this subfamily are either diurnal, crepuscular, or nocturnal, and characteristic with bright and contrasted colouration that is considered relevant to aposematism or mimicry, both unusual in Noctuidae. The larvae of Agaristinae are also bright-coloured and feed on several plant families that contain alkaloid secondary compounds. The male of several genera possess an exaggerated forewing tymbal structure which is used for sound production, and distribution of these sound-producing genera seems to imply a Gondawanan origin. In contrast with its high diversity, the studies on Agaristinae systematics and biology are fairly rare. In the present study, we present the first phylogenetic study of Agaristinae to address several questions: (1) is Agaristinae a monophyletic group? (2) is the classification system proposed by Kiriakoff (1976) approved by modern method? (3) if the taxa having swollen forewing costal margin are closely related and suggest any biogeographical significance? The phylogenetic pattern shows that Agaristinae of the current concept is monophyletic, but adult morphology may have little help in resolving the relationship because both the male and female genitalia are relatively conservative across . The "chorotaxa" system proposed by Kiriakoff, therefore, cannot be rejected due to the poor resolution of the phylogeny. Meanwhile, the taxa having protruded forewing do not constitute a monophyletic clade, so this character might have evolved independently in different continents.

Keywords:*diurnality, aposematism, Noctuidae, Phylogeny*

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O105TU02

Taxonomy

O105

Early amphiesmenopteran and lepidopteran fossils from the mid-Jurassic Jiulongshan formation of China reveal their early evolution and diversity

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The Jiulongshan Formation of Daohugou Beds is situated along the provinces of western Liaoning and eastern Inner Mongolia in China. This well known deposit has yielded an abundant, diverse and exquisitely-preserved assemblage of terrestrial fossils that provide a unique window for examining in particular the morphology and ecology of Mesozoic insects. The insect material at Daohugou especially has been important for furthering knowledge about the holometabolan lineage Amphiesmenoptera whose early evolution remains poorly known because of their depauperate Mesozoic fossil record. We have found approximately 14 specimens at Daohugou that can be assigned to the Amphiesmenoptera, representing the common ancestral stock preceding the complete divergence of Lepidoptera and Trichoptera, or otherwise are referable to the Trichoptera and basalmost Lepidoptera. We have assessed the morphologies of the Daohugou specimens and their affinities with other basal amphiesmenopteran families from the Jurassic, such as Necrotauliidae, Archaeolepiidae, Mesokristenseniidae and Eolepidopterigidae. In addition, we have explored the process of how the lepidopteran apomorphies may have evolved. The new fossils provide the earliest records of lepidopteran mandibulate mouthparts and possible internal feeding within amphiesmenopteran/lepidopteran lineage, hinting their ecological diversity. The mid-Mesozoic diversity of the Amphiesmenoptera and Lepidoptera is comparable to other insect lineages from Jiulongshan Formation whose abundance may be directly related to preservational potential, and thus taphonomic bias.

Keywords:*Amphiesmenoptera, Lepidoptera, Trichoptera, fossil, paleoentomology, Jiulongshan, China*

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O105TU03

Taxonomy

O105

Discovery of a new species of the genus *Roeslerstammia* Zeller (Lepidoptera, Roeslerstammiidae) from China, with notes on the identity of two Indian species described by Meyrick

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The genus Roeslerstammia includes four species: Roeslerstammia erxlebella (Fabricius, 1787) and R. pronubella (Denis & Schiffermuller, 1775) from Europe to Japan, and R. metaplastica Meyrick, 1921 and R. hemiadelpha Meyrick, 1922 from Punjab, India. Although some reports on morphology and biology of the former two species have been published, nothing has been known about the two Indian species described by Meyrick since the original description, and no species has yet been recorded from China. In May, 2009, we collected an unknown species of microlepidoptera at Tianpingshan, in the northern part of Hunan Province, China. As a result of examination, we confirmed the following characters which are diagnostic in Roeslerstammiidae (Kyrki, 1983): forewing R5 reaching to costa, tegumen bilobed, gnathos present, valva with a small pad of long hair scales on the outside near the base, aedeagus consisting of a slender curved tube. In the species collected in China, there is a triangular white marking at costa and the shape of the aedeagus and valva in the male genitalia is unique, which indicates that it represents a new species belonging to Roeslerstammia. On the basis of material deposited in BMNH (London), the two Indian species described by Meyrick are reviewed for the first time since the original description and the identity of the two species is also commented on.

Keywords:*Roeslerstammiidae, Roeslerstammia, taxonomy, morphology, China, India*

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O105TU04

Taxonomy

O105

Review the fauna of Pyraustinae (Lepidoptera, Crambidae) in NE China

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Pyraustinae are one of the subfamily of Crambidae belong to Lepidoptera, which comprises 7381 species in the world (Heppner, 1991). They can be identified by the following characters: Chaetosema absent, labial palpus and proboscis well developed, maxillary palpus filiform or brush-shaped; forewing with R3 and R4 stalk at base, or R2, R3 and R4 stalked, but R5 single; Cup absent, 2A and 1A form a loop; hindwing Sc+R1 stalked with Rs; male genitalia with gnathos absent or rudimentary. The Northeast region of China comprises Heilongjiang, Jilin, Liaoning Province, and Inner Mongolian, The length of NE China from south to north is 1600 km, and the total area is 1,241,400 km2, it includes Greater and Lesser Xing'an Mountains, Changbai Mountains, Zhangguangcailing Mountains, and Laoyeling Mountains. NE China is an important area for forestry and agriculture, many diseases and pests occurred every year. So the research on biodiversity of NE China is of great significance in systematic study and the basis for pests control in NE China. Up to now,there are about 700 Pyraustinae species in China (Li houhun, 2009), but only a few species reported from northeast. Accordingly, it is of great importance to survey the Pyraustinae in NE China. In this study, we indentified 102 Pyraustinae species from NE China, of them, 3species are the first time reported in China, and 11 are the first time reported in Heilongjiang Province, 16 are the first time reported in Jilin Province, and 21 are the first time reported in Liaoning Province.

Keywords:*Crambidae, Pyraustinae, fauna, new record, NE China*

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Three new species of Gracillariidae (Lepidoptera) from China and Japan, with descriptions of the pupal morphology of the genera *Eumetriochroa, Guttigera* and *Metriochroa*

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Adult Oecophyllembiinae sensu Kumata (1998) are some of the smallest leaf-mining gracillariid subfamilies, with a wing expanse of only 5-10 mm. Recent authors (Vari et al. 2002; De Prins & De Prins, 2005, 2012) treated Oecophyllembiinae as a synonym of Phyllocnistinae. Because the position of this sister-group remains unclear, we follow Kumata (1998) in placing six genera in Oecophyllembiinae, and Phyllocnistis as the sole member of the Phyllocnistinae. In China, we recorded two Oecophyllembiinae species: Corythoxestis sunosei (Kumata, 1998) and Eumetriochroa hederae Kumata, 1998, and described the pupae of the genera Corythoxestis and Eumetriochroa for the first time (Kobayashi et al., 2011). We found three diagnostic differences in them from other genera of Oecophyllembiinae and Phyllocnistis. In the present study, as a result of our field work in China and Japan, we recognize three new Oecophyllembiinae species, among which are included a Metriochroa sp. feeding on Symplocaceae and Eumetriochroa sp. and Guttigera sp. feeding on Araliaceae. Pupae of six species of the genera Eumetriochroa, Guttigera and Metriochroa are described for the first time. We also report life histories and new host plants of Corythoxestis sunosei from Japan. Pupae of Eumetriochroa species have paired setae on the clypeus, and Oecophyllembiinae species have a cremaster with two pairs of caudal processes. The pupal characters described above may provide a useful diagnosis between species of Oecophyllembiinae genera and Phyllocnistis.

Keywords:*Oecophyllembiinae, Phyllocnistis, taxonomy, morphology, China, Japan*

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O105TU06

Taxonomy

O105

Overview of moths diversity (Lepidoptera) in the Northern Part of Vietnam

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The fauna of the Moths in Vietnam is poorly known. The first data for moths of Vietnam was initiated by French entomologist, de Joannis (1900, 1912, 1928-1929), reported 527 species from the northern part of Vietnam. Kitching & Spitzer (1995) reviewed 117 species of Sphingidae, 271 species of Noctuidae were listed by Hungarians (Ronkay, 1989; Hreblay & Ronkay, 1999; Hreblay & Ronkay, 2001). In 2000, 290 species of Notodontidae, 98 species of Lasiocampidae and 153 species of Limacodidae were reported (Zolotuhin, V.V & T.J. Witt, 2000). Kuznetzov (2000) annotated a list of 208 tortricid species from Vietnam. Recently, 33 species of tortricidae were described as new to science by Razowski (2008 & 2009). Besides, moths of Vietnam was also reviewed or described by other entomologists; Prout (1921), Candeze (1927), Boursin (1928, 1940), and Lamee & Tam (1950). One of the recent Vietnamese publications on moths of agricultural pests was published by the National Institute for Plant Protection (1977-1978 & 1997-1998) with 24 species of Noctuidae, 77 species of Pyralidae, and some other moths were listed. In the present study, from 2004 to 2010 with 14 times of survey in the Northern part of Vietnam have been done by Vietnamese and Korean entomologist. Totally, 564 species of Noctuidae, 201 species of Pyralidae, 23 species of Lecithoceridae, and 28 species of Sphingidae were recorded (Park et al., 2007). Besides, more than 120 species of Tortricidae were recognized from current study.

Keywords:*Taxonomy, biodiversity, fauna, moths, Lepidoptera, Vietnam*

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O105TU07

Taxonomy

O105

Systematics of Asian skippers (Lepidoptera: Hesperiidae): What have been known and what should be done.

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The taxonomy of Asian skippers has been relied largely on a series of catalogues compiled by Brigadier W. H. Evans. Although, numerous new taxa have been added and some parts of Evans' work have been revised, his catalogue still plays a major role in the study of Asian skippers. Limitation of Evans' 6-volume work is its separation into 3 geographic regions, namely Africa, Eurasia & Australia, and America. The relationship of taxa and their pattern of distribution across these areas are not fully discussed. Taxonomic revisions of the group including Asian and African taxa are being progressed, and many, if not all, Afro-Asian genera are revealed to be polyphyletic. Current development in information technology changed such a research from a taxonomist's solitary work to collaborative research involving people far away. Recently, a framework of higher taxonomic category based on molecular and morphological phylogeny is settled. The status of the Asian skippers is not drastically altered in this new framework. However, tribal level relationship, or genus group in Evans' sense, of the subfamily Hesperiinae is not resolved satisfactorily, leaving many taxa in Incertae Sedis. Molecular data are being accumulated, notably in DNA barcoding locus. Availability of fresh specimens is the major limiting factor. Local checklists have been updated extensively. However, considerable places such as Sumatra and Java have been left behind. Evans' catalogue is unsatisfactory as an identification tool, and reliable taxonomic keys are needed. The genus Choaspes (Coeliadinae) is employed as a model case to demonstrate the current progress.

Keywords:*Hesperiidae*

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O105TU08

Taxonomy

O105

How could we solve taxonomic conflicts between traditional data and DNA barcodes in the Korean Butterflies?

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We analyzed 921 DNA barcodes (4.6 samples per species) for 202 resident Korean butterfly species. Most samples were collected from 2009 to 2011, and we obtained 28 samples of 11 species from the adjacent regions such as Russia, North Korea, and Japan for comparative analysis with the closely related species. The obtained samples were morphologically identified based on wing patterns. In a result, genetic divergence to the nearest-neighbouring taxon varied from 0 to 28.2%, with an average of 13.4 per cent. The neighbour joining (NJ) tree profile showed that sequence data for 185 (91.6%) of the 202 species formed distinct barcode clusters, allowing for their unambiguous identification. However, the rest 17 species were not matched for the traditional taxonomy. They consist of two species pairs (2.0%) that separated from each species by less than 1% branch length, two species pairs and one triple species pair (3.4%) that were paraphyletic, three species (3.0%) that were polyphyletic with sharing barcodes, and four clusters that separated from existing species by deep branch length and barcoding gap. These ambiguous cases could not be identified by only using DNA barcodes and wing patterns. Therefore, we tried to identify them using other taxonomic methods such as examination of aedeagal characters, RAPD, analysis of other gene regions, and comparison of ecological traits for each species.

Keywords:*DNA barcoding, COI, Butterfly, South Korea, Integrative taxonomy*

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O105TU09

Taxonomy

O105

Systematic study on the genus *Promalactis* Meyrick (Lepidoptera: Oecophoridae)

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The genus Promalactis Meyrick 1908 is one of the largest genera of the family Oecophoridae (Lepidoptera), comprising more than 150 species worldwide with a great diversity in the Palaearctic and Oriental regions, especially in Southeast Asia (Kim et al. 2010). More than 90 species have been reported from eastern Asia, approximately 40 species are known from Southern Asia (Kim et al., 2012). They are characterized by brownish yellow to yellowish brown ground color with variable white marking, such as oblique, median, costal, or apical spots. We focused on that Promalactis species having variable genitalia can be grouped into same or similar wing pattern. To examine the availability of wing pattern as a key character for species grouping, we analyzed the morphological data, wing pattern forms, and molecular data based on the mitochondrial COI gene. Additionally, a phylogenetic relationship within the family was conducted, based on mitochondrial COI and 16s rRNA genes, and nuclear 28s rRNA and 18s rRNA genes. As a result, we confirmed that the groups of genetically close Promalactis species on the mitochondrial COI correspond to the groups with similar wing patterns, and also grouped as the monophyletic clades. Based on these results, we propose a taxonomic importance of wing patterns as key character for the species-groups, and a new genus by unique wing pattern for Promalactis polyspina.

Keywords:*Oecophoridae, Promalactis, species grouping, morphological and molecular analyses, new genus*

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O105TU10

Taxonomy

O105

Does mimetic coloration of the model always evolve earlier than that of mimic? A case study based on the "Euploea mulciber mimicry complex" in SE Asia

Chia-Hsuan Wei[1], Li-wei Wu[2], Yu-feng Hsu[3], Shen-Horn Yen[4]

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In a mimicry system, the model possessing a remarkable pattern and secondary defense mechanism simultaneously is generally expected to have evolved earlier than the co-mimics, which benefit from the resemblance of the colour pattern. Previous studies have demonstrated that while a mimetic pattern arose from a non-mimetic pattern, population differentiation or speciation was usually attended. The relative divergence times among independent co-mimicking lineages carrying multiple defense mechanisms, however, have rarely been explored. In the present study, we focused on the "Euploea mulciber" mimicry complex characterized by having sexually dimorphic wing patterns that are perfectly matched between both sexes of co-mimicking species, which have sympatric polymorphic forms, various secondary defense mechanisms and a wide distribution range throughout E. Himalaya, Indochina and Sundaland. Sequence information from multiple genes were extracted to reconstruct the individual phylogenies of four lepidopteran groups, Euploea, Elymnias (Nymphalidae), Papilio (Chilasa) (Papilionidae), and Cyclosia (Zygaenidae). Estimates of divergence times show that the mimicry complex might have formed since middle Miocene. The estimated divergence times of Cyclosia (with chemical defense based on cyagnoglocosides) and Elymnias (without chemical defense) are later than that of Eu. mulciber. Either the genus Chilasa or the mimicking Ch. paradoxa has evolved earlier than Eu. mulciber. The results suggest that species with non-mimetic pattern can involve earlier than the model, but the mimetic pattern arises subsequently.

Keywords:*Batesian mimicry, Mullerian mimicry, chemical defense, correlated evolution, character evolution*

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O105TU11

Taxonomy

O105

Taxonomical traits of native population of *Antheraea assamensis* Helfer (Muga silkworm) of Assam, India

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The sericigenous Saturniid Antheraea assamensis Helfer, 1837, (muga silkworm) having the lowest chromosome number n=15 is known as the ancestral form of the genus Antheraea. This insect is exclusively confined to the Brahmaputra valley of Assam and some parts of neighboring states of NE region of India due to its unique ecological niche. Although it is a monotypic species, it has four distinct larval color morphs, namely green, blue, orange and yellow and these are non diapausing multivoltine in nature. A diapausing wild morph is also found in the forests of this region. Lack of systematic study on taxonomical trait of this natural population instigated to study the distinguished morphological characters in egg, larva, cocoon and moth stages. During the study negligible variation was observed among the color morphs and there is no impact of larval coloration on cocoon characters. However significant variation was observed in respect of voltinism, geotropism, chorion structures, larval tubercle, setae number, cocoon characters, wing pattern, male genitalia and antennae between the color morphs and the wild morph. Based on this study the three color morphs viz. green ,blue and orange could be clubbed together and the wild morph remains separately .It is expected that this study will help in designing the breeding programme for improved hybrid vigor as the population is suffering from inbreeding depression. It will help in maintaining the stable and sustainable population, thereby saving the precious germplasm of A. assamensis from extinction.

Keywords:*Taxonomical traits, Native population, Antheraea assamensis, morphs, germplasm*

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O105TU12

Taxonomy

O105

Arctiid Diversity from India

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The moths belonging to family Arctiidae of order Lepidoptera is an attractive and fascinating group of insects in terms of species diversity and also of immense economic importance. These moths are colorful creatures, often with moderately broad wings which are conspicuously spotted, banded or otherwise marked with bright colors. Over 4000 species are known from the globe and group is well represented in nearly all zoogeographical regions but attains its greatest development in the tropics. Arctiid moths are defined principally by the presence of tymbal organ on metaepisterum and pre- spiracular counter tympanal hood. Studies on taxonomy of Indian Arctiid moths is in progress for the last two decades. As many as 512 species of these insects are known from India and out of these 356 species have been collected, studied and authentically identified. Taxonomic revision of Indian Arctiidae has brought many new taxa to light. Collection-cum-survey tours have been conducted in the far flung localities of India to study these beautiful moths. External gentialic structures of all the studied species have been dealt with and illustrated in detail. A lot of nomenclature changes have been suggested for the future workers. The present communication deals with Arctiid diversity from India and the importance of external genitalic structures in the taxonomy of this family has been highlighted.

Keywords:*Arctiid, Moths, Diversity, Gentialia*

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O105TU13

Taxonomy

O105

Composition of subfamily Tortricinae (Lepidoptera, Tortricidae) from Northern Part of Vietnam

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Tortricinae is the largest of three subfamilies in the Tortricidae and was divided into 11 tribes (Horak and brown, 1991). Tortricinae can be distinguished from other subfamilies by the two rings of scale on each antenna segment, and the caulis articulated with the juxta by a short membrane. About 1010 named species are presenting in Palaearctic region (Razowski, 2008) including 382 species in Europe (Razowski, 2003), 260 species were recorded from South East Asia (Robinson, Tuck and Shaffer, 1994). The fauna of Tortricinae in Vietnam is poorly known even some expeditions were previously done by some foreign entomologist. One of the recent Vietnamese publications on moths of agricultural pests was published by the National Institute for Plant Protection (1977-1978 & 1997-1998), only seven Tortricidae species were recorded. In the year, 2000 Kuznetzov annotated a list of 208 tortricid species from Vietnam. Recently, more than 80 species belong to Tortricinae are announced from 2008 to 2009 by Razowski. Of them, 33 species were described as new to science. At present, the tortricid fauna of Vietnam is estimated approximately 315 species (Svetlana V. Nedoshivina, 2010). From the results of the study on subfamily Tortricinae in Northern part of Vietnam, totally, 28 species of 13 genera are recognized, but only 9 species were previously recorded. Besides, some unidentified species are waiting for further study. The materials examined are based on the collection of University of Incheon, which were collected from 2004-2010 in the Northern part of Vietnam by Vietnamese and Korean entomologist.

Keywords:*Lepidoptera, Tortricidae, Tortricinae, composition, Vietnam*

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O106TH01

Taxonomy

O106

Finding the sister group of Cryptocercidae + Isoptera: A combined molecular and morphological phylogeny of Dictyoptera

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Recent phylogenetic studies have established that termites belong within the cockroaches as sister to Cryptocercidae. Cryptocercus shares several important behavioural traits with termites, thus we need to understand the phylogenetic position of Cryptocercidae + Isoptera to determine how these traits evolved. However, placement of Isoptera + Cryptocercidae is still uncertain. Suggested sister groups include Polyphagidae + Lamproblattidae, Polyphagidae + Nocticolidae, Blattidae, Tryonicidae, Blattidae + Tryonicidae, and all other Dictyoptera. None of the possible sister groups are known to share more similarities in lifestyle with Cryptocercidae + Isoptera than the others; e.g. representatives of all have been found in rotting wood. Previous phylogenetic studies of Dictyoptera have been either molecular or morphological. The only exception is Ware et al. (2008) using already published data resulting in a large proportion of missing data, and no targeted taxon sampling. Despite the large number of studies, no consensus has been reached on the deep splits within Dictyoptera. The present study includes all the previously suggested sister groups of Isoptera + Cryptocercidae as well as other potentially important taxa such as an Australian polyphagid which form colonies in wood cavities, but have never been included in phylogenetic studies. We combine molecular and morphological characters as well as a large outgroup sampling to produce a stable phylogeny of Dictyoptera. Finding the sister group of Cryptocercidae + Isoptera offers insight into the development of an ecologically essential group which can also serve as a model for development of eusociality.

Keywords:*Phylogeny, Dictyoptera*

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O106TH02

Taxonomy

O106

Molecular and phylogeographic analysis of the relict dragonflies of *'Epiophlebia'* (Insecta: Odonata)

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The relict dragonflies of 'Epiophlebia' show several peculiar traits, e.g. in their disjunct distribution in the Eastern Palearctic and Oriental regions as well as in the unique combination of morphological characters, which are otherwise characteristic of dragonflies or damselflies. The three extent species 'Epiophlebia superstes' Selis, 1889, 'Epiophlebia laidlawi' Tillyard, 1921 and the lately discovered 'Epiophlebia sinensis' Li & Nel, 2011 are the only recent representatives of these strongly cool-adapted dragonflies. Our results gain insights into the interspecific similarities in the DNA of the 'Epiophlebia'-species, which is significantly higher than between other closely related species of Odonata. It resembles the degree of similarity found in different populations of the same species. Furthermore, the morphological differences between of 'E. laidlawi' and 'E. superstes' are minor and might not be sufficient for taxonomically maintaining three species in the genus 'Epiophlebia'. Therefore, we float a suggestion that the occurrences of 'Epiophlebia' in the Himalaya probably are populations of 'Epiophlebia superstes'. Such a disjunct distribution reflect historical events, and a major goal of research is to deduce which events were responsible for the given distribution pattern. The minor differences in the DNA sequences indicate that the extant population of 'Epiophlebia' had a significant genetic exchange until quite recently. The last possibility for such an exchange most likely existed during the last ice age when the sea level was at its lowest approximately 20000 year ago. At this time the area occupied by 'Epiophlebia' probably was much larger, encompassing and connecting its extant populations.

Keywords:*Odonata, Phylogeography, glacial relicts, 'Epiophlebia', DNA homogeneity*

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O106TH03
Taxonomy

O106

The evolution of insect flight

Thomas Hoernschemeyer
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The ability to fly is essential for the evolutionary success of the winged insects, which make up more than half of all known species. Nevertheless, the origin of insect flight, its morphological foundations and the subsequent evolutionary events are still not well understood. One aspect that probably contributes significantly to our problems in deciphering the processes in the evolution of insect flight is the considerable age of this trait. Already as early as in the middle of the Carboniferous, approximately 324 million years ago, pterygote insects with a fully developed flight apparatus existed. This indicates that the group had its origin significantly earlier, perhaps in the lowest Carboniferous or even in the upper Devonian. Investigations of the morphology of extant pterygotes, of their embryological development and of insect fossils had previously resulted in several contradicting hypotheses on different aspects of the origin and evolution of insect flight. Now, our recent comprehensive studies of the flight apparatus morphology, including musculature and sclerites, of numerous representatives of Ephemeroptera, Odonata and Neoptera, supplemented with investigations of the ontogenetic development of these structures and combined with the reanalysis of insect fossils allow for new interpretations of several aspects in the sequence of events of the evolutionary origin and development of insect flight.

Keywords:*Ephemeroptera, Odonata, Neoptera, flight, evolution, morphology, fossil*

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O106TH04

Taxonomy

O106

A preliminary phylogeny of Pyrgomorphidae (Orthoptera: Caelifera) based on morphological characters

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Pyrgomorphidae is the sole member of the superfamily Pyrgomorphoidea within Acridomorpha (Orthoptera: Caelifera). Commonly known as the gaudy grasshoppers, pyrgomorphs are easily diagnosable by a groove in the fastigium and phallic structures, but there are great morphological variations within the family that has led to 140 years of taxonomic instability. Currently the family is composed by 477 species distributed mainly in the Old World as well as Mexico, South America, and Australia and is divided in two subfamilies, Pyrgomorphinae and Orthacridinae, which include 31 tribes and 5 genera incertae sedis. The most recent phylogenetic analysis based of mitochondrial genome data places Pyrgomorphidae as sister to Acridoidea. In this study, we reconstruct a preliminary morphological phylogeny of Pyrgomorphidae based on 40 ingroup genera representing 10 of the 13 tribes from the subfamily Orthacridinae and all the 18 tribes from the subfamily Pyrgomorphinae. This is the first attempt to obtain a comprehensive phylogeny of the family that will provide new insight into understanding the evolution of this interesting family.

Keywords:*Pyrgomorphidae, Orthoptera, Phylogeny*

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O106TH05

Taxonomy

O106

The phylogeny of the holometabolous insect orders inferred from transcriptomic, genomic, and morphological data

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Holometabola (= Endopterygota) is one of the most species-rich lineages of living organisms. Extensive efforts have been made to reconstruct its phylogeny. However, the evolution of the group is far from being completely understood. For example, the phylogenetic relationship of twisted-wing parasites (Strepsiptera) to other endopterygote insects has proven notoriously difficult to resolve ("The Strepsiptera problem"). To infer a robust backbone tree of the holometabolous insect orders, we (1) collected comprehensive transcriptome data, (2) sequenced the genome of a twisted-wing parasite, and (3) compiled extensive morphological data sets. The transcriptome data include for the first time representatives of all recognized holometabolous insect orders. In all major lineages we selected species for sequencing that diverged early in the evolution of each order and that can be assumed to be plesiomorphic in many aspects. The genome sequence data of Mengenilla (Strepsiptera) include more than 1.8 million sites of orthologous and inparalogous genes plus sequence-independent genomic meta-characters, such as gene order and the position of introns within genes. The morphological data encompass characters of different life stages and body parts, including many internal features. The analyses of these data allowed a solid reconstruction of the interordinal relationships of holometabolous insects, including the clarification of the phylogenetic origin of the Strepsiptera. We found overwhelming support for Strepsiptera being the sister group of monophyletic beetles. The inferred phylogenetic tree of holometabolous insects facilitates a better understanding of the evolution of morphological characters and of developmental and ecological traits.

Keywords:*Holometabola, Phylogenomics*

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O106TH06

Taxonomy

O106

Early evolution and historical biogeography of fishflies (Megaloptera: Chauliodinae): Implications from a phylogeny combining fossil and extant taxa

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Fishflies (Corydalidae: Chauliodinae) are one of the main groups of the basal holometabolous insect order Megaloptera, with ca. 130 species distributed worldwide. A number of genera from the Southern Hemisphere show remarkably disjunctive distributions and are considered to be the austral remnants or "living fossils" of Gondwana. Hitherto, the evolutionary history of fishflies remains largely unexplored due to limited fossil record and incomplete knowledge of phylogenetic relationships. Here we describe two significant fossil species of fishflies, namely Eochauliodes striolatus gen. et sp. nov. and Jurochauliodes ponomarenkoi Wang & Zhang, 2010 (original designation for fossil larvae only), from the Middle Jurassic of Inner Mongolia, China. These fossils represent the earliest fishfly adults. Furthermore, we reconstruct the first phylogenetic hypothesis including all fossil and extant genera worldwide. Three main clades within Chauliodinae are recognized, i.e. the Dysmicohermes clade, the Protochauliodes clade, and the Archichauliodes clade. The phylogenetic and dispersal-vicariance (DIVA) analyses suggest Pangaean origin and global distribution of fishflies before the Middle Jurassic. The generic diversification of fishflies might have happened before the initial split of Pangaea, while some Gondwanan-originated clades were likely to be affected by the sequential breakup of Pangaea. The modern fauna of Asian fishflies were probably derived from their Gondwanan ancestor but not the direct descendents of the Mesozoic genera in Asia.

Keywords:*Megaloptera, fossil, new taxa, phylogeny, biogeography*

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O106TH07

Taxonomy

O106

Phylogenetic analysis of Panorpidae (Mecoptera) with special reference to the female genital plates

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Male genitalia generally exhibit species-specific morphology and have been widely used in taxonomy and phylogenetic analysis in many groups of insects. However, for the simpler and less varied female genitalia in most insects, the significance has been considerably overlooked or neglected in the phylogenetic analysis. Panorpidae are the largest family in Mecoptera, comprising ca. 420 species, and their phylogeny has been not investigated hitherto. Panorpa has been confirmed paraphyly for a long time. The diverse species of the Chinese Panorpa are categorized into four species groups [the P. amurensis, P. centralis (=P. cornigera), P. diceras and P. davidi groups]. The female genital plates of 26 species in four genera of Chinese Panorpidae were investigated using light and scanning electronic microscopy. The phylogenetic relationship was analyzed based on the female genital plates as well as other characters with Brachypanorpa carolinensis and Panorpodes kuandianensis as outgroups. The result shows that the female genital plate could provide reliable phylogenetic information for Panorpidae, especially valuable at generic level. In the cladogram, Sinopanorpa is evidently monophyletic. Neopanorpa is paraphyletic with Leptopanorpa. Panorpa is paraphyletic with both (Neopanorpa + Leptopanorpa) and Sinopanorpa. The P. amurensis, P. centralis, and P. diceras groups are evidently monophyletic and might display a potential generic status, respectively. The P. davidi group is very likely to be paraphyletic and need to further study. The origin and evolutionary pathway of the female genital plate in Panorpidae is briefly discussed.

Keywords:*Panorpidae, female genitalia, morphology, phylogeny*

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O106TH08

Taxonomy

O106

Characteristics of genome sequence of "*Candidatus Hamiltonella defensa*", an endosymbiont of the whitefly, *Bemisia tabaci*

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"Candidatus Hamiltonella defensa" is a maternally transmitted endosymbiont found sporadically in some sap-feeding insects. In some aphid individuals, the facultative symbiont Hamiltonella provides protection against parasitoid wasps by causing high mortality of wasp larvae, or induces the expression of host heat shock genes to increase host heat tolerance. In whitefly Bemisia tabaci complex, Hamiltonella is found in two important invasive cryptic species, MED and MEAM1. Herein, we report the properties of the draft genome sequence of Hamiltonella from an invasive whitefly, the MED cryptic species. The adequate bacterial DNA was obtained by whole genome amplification and then was sequenced using the Illumina HiSeq 2000. The paired-end reads were de novo assembled with SOAPdenovo v1.05. The coding sequences were predicted by Glimmer v3.0, and homologous comparison to a nonredundant public database was performed by BLAST for functional annotation. Finally, we obtained the draft genome of Hamiltonella, which has approximately 1.84 Mbp size with low GC content (40.3%) and comprises 404 contigs. Totally, 1,806 genes are predicted within the genome of Hamiltonella. Out of these genes, 1,112 were mapped to the pathways in KEGG, from which 'Membrane Transport' (n=301) has the highest percentage of genes followed by 'Replication and Repair' (n=227), 'Translation' (n=136), 'Carbohydrate Metabolism' (n=128), 'Metabolism of Cofactors and Vitamins' (n=88) and 'Amino Acid Metabolism' (n=87). These annotations provide a valuable resource for investigation of the functions of Hamiltonella in whitefly. Further genomic analyses and comparative studies will give additional insights into this symbiosis-host system.

Keywords:*Bemisia tabaci, symbiont, Hamiltonella, genome*

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O106TH09

Taxonomy

O106

Comparative analysis of mitochondrial genomes in Diplura (Arthropoda: Hexapoda) and its utility in Diplura phylogeny inference

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Diplura is one of the most important groups of basal hexapods. There are three major lineages in Diplura: Campodoidea, Projapygoidea and Japygoidea. The monophyly of Diplura has been debated because Campodeoidea and Japygoidea differ in the type of cerci and ovarian structure. Some phylogenetic analysis based on molecular markers also do not support Diplura is a monophyletic clade. However, few studies have included Projapygoidea, whose phylogenetic position within Diplura is still a puzzle. Up to the present, there has been a noticeable lack of mitochondrial genome data from Projapygoidea. In this study, we determined and annotated the complete mitochondrial genome of Octostigma sinensis (Projapygoidea: Diplura), as well as three other new Diplura mitochondria genomes, one from Campodoidea and two from Japygoidea. All four newly sequenced together with three reported mitochondrial genome of diplura encode 13 protein-coding genes, 22 transfer RNAs and two ribosome RNA genes, and bear the same gene order as the pattern shared by most crustaceans and hexapods. Structural changes have occurred in trnR, trnC, trnS1 and trnS2 as previously reported, and reduction of tRNA arms were taxonomically correlated. The phylogenetic analysis based on 12 mitochondrial protein-coding genes (without nad4L) suggested that Diplura was a good monophyletic group and Projapygoidea was more closely related to Campodoidea than Japygoidea. We also noticed that in the phylogenetic analysis based on nucleotide information of protein-coding genes, the monophyly of Diplura cannot be recovered when Octostigma sinensis were excluded. This suggested that the lack of key taxon might get a wrong conclusion.

Keywords:*Diplura, mitochondrial genome, Hexapoda, tRNA reduction*

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O106TH10

Taxonomy

O106

Systematics of the Australian triozid fauna (Hemiptera: Psylloidea) from the Casuarinaceae

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The triozid fauna of the Casuarinaceae is being used as a model system to determine whether insect herbivores and their hosts have co-speciated, providing a molecular basis for the selection of suitable biological control agents. It is hypothesised that phylogenies synchronous between herbivore and host would indicate a close evolutionary association, and therefore high host specificity and safer biological control options than a group that may have radiated on multiple host taxa. The phytophagous jumping plant-lice, or 'psyllids', appear highly host-specific, making them excellent candidates for co-phylogenetic studies. Most species are monophagous, few are oligophagous, and rarely polyphagous. Closely-related psyllids occur on closely related hosts, mostly recorded from single plant families. The triozid 'psyllid' fauna on Casuarinaceae is being revised: Aacanthocnema (6 spp.), Acanthocasuarina (6 spp), Trioza (4 spp.) and an undescribed genus (16 spp.) occurs on Allocasuaraina, and Casuarinicola (5 spp.), together with an additional undescribed genus (5 spp.) on Casuarina. Mapping of host data indicate two independent host switching events from Allocasuarina to Casuarina in the radiation of the Australian triozid fauna, and one of the gall-forming Schedotrioza to Eucalyptus. There is both a high generic-level and species-level host specificity between the triozids and their hosts suggesting their potential as biological control agents for species of Casuarina that are considered serious environmental weeds.

Keywords:*co-evolution, biological control*

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O106TH11

Taxonomy

O106

Phylogeny of Stylops (Strepsiptera) reveals the way of speciation through host specialization to host *Andrena* bees

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Genus Stylops is a member of enigmatic insect order Strepsiptera. Their taxonomy or even ecology and other details about their biology are poorly known. Males are known for their extraordinary short life (just about five hours) and females are neotenic and all their life bound to the host body. We prepared DNA sequences for Stylops females from wide range of host species from Africa, Asia, Europe as well as North America. Based on these sequences, we constructed a phylogenetic tree to test the hypotheses of Stylops speciation. According to published literature, there are three different concepts: 1) every species of host Andrena possess its own specialized Stylops parasite; 2) every subgenus of host Andrena has its own particularly specialized Stylops parasite; 3) there is a single generalistic Stylops parasite. We discovered a wide range of biological variability, which probably came from coevolutionary history of every lineage of Stylops and its host Andrena species. Just the third hypothesis can be definitely rejected. We also provide several different views of possible mechanisms of speciation in this wonderful parasite species.

Keywords:*Strepsiptera, phylogeny, host-parasite coevolution, speciation*

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O106TH12

Taxonomy

O106

Preliminary phylogeny of early lineages of scale insects (Hemiptera: Coccoidea) based on adult male morphology from the early Cretaceous to recent

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Scale insects have profound significance in agriculture, forestry, and horticulture, which has necessitated a sophisticated species-level taxonomy. Phylogenetic work, however, lags behind taxonomy, particularly with regard to families that have traditionally been grouped into the probably paraphyletic "archeococcoids." This has also compromised the interpretation of the very diverse palaeofaunas of the Coccoidea, such as those beautifully preserved in amber from the Neogene to the Early Cretaceous (ca. 20-130 myo). Indeed, most fossil coccoids are winged males, but the taxonomy of Recent scales is based largely on the wingless, highly reduced females, further compromising fossil interpretation. Fortunately, the study of Recent adult males has increased greatly, thus allowing them to be incorporated into phylogenetic research. We report here the phylogenetic results of our work using males of exemplar Coccoidea through the past 130 million years to the present. A comparison is made of the chronology of coccoid divergence times with major events in earth history, such as the radiation of the angiosperms.

Keywords:*amber, Coccoidea, adult males*

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O106TH13

Taxonomy

O106

Cladistic analysis of Caelifera based on continuous-discrete morphological data

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The phylogenetic relationships among some taxa within the suborder Caelifera (Orthoptera) were unclearly until now. In this study, 17 genus and species belonging to four superfamilies, three families, eleven subfamilies and two tribes were used as sample taxa and their phylogeny was studied using 56 discrete and 41 continuous morphological characters combined by simultaneous method. Also the mantid Iris oratoria (Mantidae: Dictyoptera) was selected as outgroup and Parsimony analyses were conducted via TNT Ver. 1.1 without weighting of any of the characters. The robustness of the clades was examined via bootstrapping test (1000 rep and 50% Del). The single most parsimonious (shortest-length) tree obtained was supported monophyly of Acridini and Truxalini tribes and also Tridactyloidea and Tetrigoidea superfamilies with 100 robustness. Also the results shows that the subfamilies Teratodinae, Calliptaminae and Eyprepocnemidinae that are traditionally classified within the Acrididae are grouped together in a clade that is named here as Teratodidae. Moreover, based on Dericorythidae and Cyrtacanthacridinae as sister group with high robustness is proposed to consider the Dericorythidae as subfamily Dericorythinae. In this evolutionary tree 2 subfamilies Pamphaginae and Tropidaucheninae are supported as a sister group with 97 robustness.

Keywords:*Phylogeny, Parsimony, Caelifera, Continuous-Discrete morphological data*

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O106TH14
Taxonomy

O106

CalBug: Digitizing California terrestrial arthropod collections

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Natural history collections house over a billion insect specimens worldwide collected over several centuries. Specimen labels encode data denoting species, location, and date captured used to study biogeographic patterns, spread of invasive species, and responses to environmental change. However, access to data is impractical for most of the research community. Because of enormous collection sizes, entomology has lagged behind other disciplines in digitizing collections. In 2010, the Essig Museum began a collaborative five-year project among eight California museums with a goal to digitize and geographically reference over one million specimens from target groups and localities. A major goal is streamlining data capture while maintaining data integrity and specimen safety. Digital imaging of labels reduces the need for repeated handling of specimens, allows enlargement of difficult to read text, and enables outsourcing of data transcription. Transcription is aided by a web-based citizen science program through collaboration with the Citizen Science Alliance. Data from each label are keyed by multiple volunteers and compared for consistency. Data are vetted in-house, normalized for variations in locality descriptions, then repatriated to an online, open-access MySQL data cache. Georeferencing of localities will occur later using semi-automated services, such as BioGeomancer, following standard protocols. The greatest bottleneck in digitizing collections is the handling of individual specimens and labels. Using high-efficiency workstations labels are removed from pins, digitally imaged with unique identifiers, repinned, and returned to their trays. Annotation of images and naming of files are done with batch processing software. Other innovations and progress-to-date will be discussed.

Keywords:*digitization, biodiversity, biogeography, database*

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O106TH15
Taxonomy

O106

Orthoptera of the Pan-Japan sea area: Taxonomic diversity, distribution and origin

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The region around the Sea of Japan include the basin of Amur River in China (provinces Heilongjiang and Jilin) and Russian Far East, Korean Peninsula, Sakhalin Island, South Kuril Islands and main islands of Japan (without Ryukyu). Totally 441 species (or 470 nominal taxa including species and subspecies) from 149 genera in 33 subfamilies and 16 families of Orthoptera are recorded from this area. Beside them 249 species in 114 genera are known from mainland and 310 species in 122 genera recorded from islands. The fauna of Orthoptera of the Pan-Japan Sea area generally is characterized by dominance of endemic to this region species, the sum of which is 65 % of the whole fauna. East-Palaearctic (18 %) and Oriental-Palaearctic species (10 %) are also common, but the sum of Afro-Eurasian, Australian-Palaearctic and Holarctic species is only 7 %. The patterns of distribution and diversity of the Orthoptera that we see today are closely associated with the history of the Sea of Japan since the Miocene. But the modern fauna of Orthoptera were basically formed during the course of the Late Wurm regresses and subsequent Holocene transgressions. The sea level in the Late Wurm climatic minimum fell some 130 meters, resulting in land bridges between Asian mainland and islands. At the end of the Wurm, the climate warmed once again, resulting in a rise of sea level. During the Holocene climatic optimum, there was a rather good chance for migration of thermophilic species of Orthoptera to the north.

Keywords:*Orthoptera, fauna, East Asia*

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O106TH16

Taxonomy

O106

Cryptic termite species diversity in Kakamega Forest, Kenya

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Termite workers are morphologically rather uniform, and thus exhibit only few traditional taxonomic characters for species identification and yet they dominate in ecological surveys. Therefore, the diversity of termites is poorly understood, especially in tropical forests which are hotspots of biodiversity and threatened by disturbances. Recently, the use of DNA- barcoding has become more important for inventory and biodiversity assessment of hyperdiverse taxa and those which are difficult to identify. An approach towards establishing a DNA barcode library for termite species identification and biodiversity assessment using sequences of the mitochondrial COII gene is presented in this study. Kakamega forest is the remnant of the Congo-Guinean Forest reaching Kenya. This isolated forest is a hotspot of biodiversity which is threatened by the increasing human population. Hence, to interpret the role of termites in different land-use habitats, the species composition needs to be understood. Here we present termite biodiversity assessment using sequences of the mtCOII gene. At least 16 termite species were observed in Kakamega Forest by morphological determination whereas at least 22 species (MOTUs) were found by molecular species delimitation.All species assessed were belonging to the family Termitidae (higher termites), and among them the fungus-growing Macrotermitinae were most prevalent with at least eight putative species, which were all cryptic within their three different genera (Microtermes, Pseudacanthotermes and Odontotermes). This highlights the advantage that molecular based species delimitation and reveals some morphological cryptic species.

Keywords:*DNA barcoding, species delimitation, COII, diversity, termites, MOTU*

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O201M01

Physiology

O201

Immune inducible factors in the cuticle of the silkworm, *Bombyx mori*

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Insect cuticle is a non-cellular matrix composed of proteins, carbohydrates and chitins. The cuticle is often regarded as just a "physical armor". However, it contains water, which enables biochemical reactions to occur, and various types of defense systems, such as anti-microbial peptides and melanization cascade, are found in the cuticle matrix. To find novel factors involved in defense reaction in insect cuticle, proteomic analyses were performed using larvae of the silkworm, Bombyx mori. Suspension of Gram-positive bacteria was injected into the hemocoel of the fifth instar larvae, and after 24 hours cuticle proteins were extracted with acidified buffer. The proteins were subjected to 2D-PAGE, and the pattern of electrophoresis was compared with that of the control larvae. Proteins, the amounts of which were increased after bacterial injection, includes antibacterial peptides, components of prophenoloxidas cascade and other molecules that have already been mentioned about their possible involvements in defense reactions in the hemolymph. Some proteins, which are candidates of novel immune inducible factors, are assumed to exhibit epithelial expressions, considering the expression patterns of the Drosohpila orthologs. The Drosophila mutants of these possible "cuticle specific" factors exhibit excess melanization reactions that might be caused by abnormal regulation of melanization cascade or increased infection. The weakened cuticle structures leading higher probability of wound can be also the reasons of the excess melanization. The relationship between the molecular functions of the factors and their possible involvements in defense reaction in the cuticle is discussed.

Keywords:*cuticle, defense reaction*

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O201M02

Physiology

O201

The characterization of a concentration-sensitive α-adrenergic-like octopamine receptor found on insect immune cells and its possible role in mediating stress hormone effects on immune function

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Octopamine (OA), the insect equivalent of norepinephrine, links the nervous system and immune system in insects. This study examines the underlying molecular mechanisms (i.e. second messenger systems) mediating OA effects on insect immune cells. At low concentrations (< 1 μM), OA stimulated hemocyte spreading and phagocytosis in the larval Lepidopteran (caterpillar) Chilo suppressalis, whereas at high concentrations (> 10 μM), OA inhibited hemocyte spreading and phagocytosis. Similarly, OA concentration had differential effects on two intracellular signaling pathways, Ca2+ and cAMP. Low concentrations of OA increased intracellular Ca2+, but only high concentrations of OA (>1 μM) led to an increase in both Ca2+ and cAMP. We identified an α-adrenergic-like octopamine receptor in this species (CsOA1) and confirmed that it is expressed in hemocytes. After heterologous expression in HEK-293 cells, the CsOA1 receptor produced the same OA concentration-dependent responses on intracellular Ca2+ and cAMP as had been observed in hemocytes. These findings support earlier work showing that OA has both stimulatory and suppressive effects on immune responses, depending on the OA concentration. Our evidence suggests that these biphasic effects are mediated by an octopamine receptor signaling through intracellular Ca2+ and cAMP second messenger pathways. Stress hormones/neuromodulators have complex effects on immune function in animals across phyla. This complexity may be mediated, in part, by conserved connections between adrenergic-like G-coupled protein receptors and second messenger systems.

Keywords:*Biogenic amine, Cellular immunity, G-protein coupled receptor, Octopamine, Neuroimmunity*

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O201M03

Physiology

O201

Hemomucin, an O-glycosylated protein on embryos of wasp *Macrocentrus cingulum* protect them from being encapsulated by hemocytes of host *Ostrinia furnacalis*

Jian Hu[1], Shengfeng Hu[2], Qiuyun Xu[3], Wenqing Zhang[4]

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Hemomucin, an O-glycosylated protein on embryos of wasp Macrocentrus cingulum protect them from being encapsulated by hemocytes of host Ostrinia furnacalis Jian Hu, Shengfeng Hu, Qiuyun Xu, Wenqing Zhang State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China Abstract Dozens of embryos of M. cingulum, a polyembryonic parasitoid may grow and develop in host's hemocoel, but how they avoid the immune reaction from host hemocytes is still unclear. In this study, hemomucin in M. cingulum (McHem) was proven to protect wasp from being encapsulated by host hemocytes. McHem is a transmembrane protein with a typical mucin domain containing 51 O-glycosylation sites and specifically conjugated with Helix pomatia lectin. Q-PCR and western blotting results indicated that McHem expressed highly in eggs, morulae and secondary embryos, but less in larvae and adult. Immunohistochemistry analysis showed that McHem mainly located on the surface of extra embryonic membrane. When the secondary embryos were transplanted into unparasitized host larvae, they proliferated into dozen of embryos, however, after the wasp's embryos were incubated with the anti-McHem serum in vitro and then were transplanted, they will not proliferated and more than 90% of them were encapsulated by host hemocytes ($p<0.01$). Furthermore, cultured with dsRNA of McHem added Ex-cell 420 medium in vitro and then transplanted, much more wasp embryos were encapsulated by host hemcoytes compare to the embryos cultured in dsGFP added medium ($p<0.01$). All these results indicated that hemomucin should play an important role in M. cingulum avoiding host's cellular immune reaction.

Keywords:*Hemomucin,Glycoprotein,immune evasion, hemocytes, parasitoid,*

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O201M04

Physiology

O201

Cytolytic activity in the mosquito, *Armigeres subalbatus*

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Although cytolysis of invading organisms is an innate form of immunity used by invertebrates, so far the underlying mechanism remains less explored. The pupal hemolymph of the mosquito Armigeres subalbatus induces an activity that causes hemolysis of human red blood cells (HRBC). This hemolytic activity was inhibited by sialic acid (N-acetylneuraminic acid) and serine protease inhibitors. We purified the sialic acid-specific lectin(s) from the pupal hemolymph using formaldehyde-fixed HRBC and determined the sequence of the amino-terminal 19 amino acid residues. A polyclonal antibody produced against this N-terminal peptide clearly inhibited the hemolytic activity of the hemolymph in vitro, thus suggesting that the hemolysis of HRBC is caused by the lectin present in the mosquito hemolymph. We suggest that mosquitoes possess a cytolysis system.

Keywords:*mosquito, insect immunity, lectin, cytolytic activity*

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O201M05

Physiology

O201

Venomic studies of an endoparasitoid *Pteromalus puparum*

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The relationships between parasitoids and their insect hosts have attracted much attention. Females of the gregarious endoparasitoid Pteromalus puparum (Hymenoptera: Pteromalidae) inject only venom along with eggs into their hosts. Here we report the venomic results of P. puparum. To facilitate research on P. puparum, we presented a method for de novo assembly of the transcriptomes of venom gland and the rest of wasp body, respectively, using short read sequencing technology (Illumina). In parallel, proteins from pure venom were separated and individually identified by LC-MS/MS analysis or reversed phase HPLC combined with N-terminal sequencing methods, compared with transcriptomic data and the non- redundant from public database. About 60% of identified venom proteins were specifically expressed in venom gland and secreted into the venom reservoir, according to the results of comparatively transcriptomic analysis between venom gland and the rest of wasp body. A set of potentially bioactive components identified from this parasitoid venom were recombinantly expressed and then the immunosuppressively functional studies of the recombinant proteins on the pupal host P. rapae were performed. Our findings support our hypothesis that P. puparum venom is a complex substance with a large number of components regulating host immune responses. We conclude that the actions of venom on host biology in ways that benefit the development and emergence of the next generation of parasitoids.

Keywords:*Pteromalus puparum, Pieris rapae, venom, composition, immunosuppression*

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O201M06

Physiology

O201

Identification and characterization of the *Diadegma fenestrale* and *D. fenestrale ichnovirus* (DfIV)

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The Diadegma fenestrale was known as parasitoid on potato tuber moth, Phthorimaea operculella and diamondback moth, Plutella xylostella. This species, genus Diadegma are first reported from Korea. Diadegma fenestrale polydnavirus; ichnovirus (DfIV) observed in D. fenestrale calyx. DfIV showed typical ichnovirus shape which two membranes surround virus capsids. The genome contents of DfIV consist about twenty-three double-stranded DNA segments ranging 1.8 to 12 kb. Total DfIV genome size was estimated to be about 115kb by adding all segment size. To identify DfIV genes, whole genome sequencing based on GS-FLX was conducted using purified total DNA extracted from D. fenestrale calyx. About sixty ORFs were analyzed and several typical polydnavirus gene family detected such as cys-motif, rep, vinnexin and vankyrin. This is the first report of DfIV and these lepidopteran host immune suppression genes and ecological characteristics will be deeply identified.

Keywords:*Diadegma fenestrale, polydnavirus, ichnovirus, DfIV, Phthorimaea operculella, Plutella xylostella*

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O201M07

Physiology

O201

Pattern recognition receptors acting in innate immune system of shrimp against pathogen infections

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Invertebrates only have innate immunity, and pattern recognition is their main strategy for pathogen discrimination. Much work has been performed on the innate immunity of the shrimp, especially on immune recognition, due to its great economic value. These studies revealed the recognition mechanism of shrimp at a certain degree. To date, 10 types of pattern recognition receptors (PRRs) have been identified in shrimp, namely, beta-1, 3-glucanase-related proteins, beta-1, 3-glucan-binding proteins, C-type lectins, scavenger receptors, galectins, fibrinogen-related proteins, thioester-containing protein, Down syndrome cell adhesion molecule, serine protease homologs, and trans-activation response RNA-binding protein (TRBP). A number of PRRs have been functionally studied and have been found to have diffident binding specificities and immune functions. This work was supported by the National Natural Science Foundation of China (Nos. 31130056, 30770282) and the Ph.D. Programs Foundation of the Ministry of Education of China (No. 20110131130003).

Keywords:*Shrimp, pattern recognition receptors, innate immunity, microbe-associated molecular patterns*

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O201M08

Physiology

O201

A novel polydnaviral gene family, BEN, and its immunosuppressive function in larvae of *Plutella xylostella* parasitized by *Cotesia plutellae*

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A full genome sequence of the episomal form of Cotesia plutellae bracovirus (CpBV) suggests eleven BEN family genes. This study analyzed their expression and physiological function in the viral host, Plutella xylostella. All eleven BEN family genes were expressed during entire parasitization period of P. xylostella larvae. In addition, these BEN family genes were expressed in fat body, gut, epidermis, and hemocytes in final larval instar of parasitized P. xylostella. The eleven BEN family genes were transiently expressed in nonparasitized larvae by injection of each viral segment containing its corresponding BEN family gene. The transient expression of BEN family genes significantly suppressed hemocyte nodule formation in response to bacterial challenge. Subsequent injection of double-stranded RNA specific to each BEN family gene suppressed the expression of the BEN family gene and rescued the immunosuppression. We propose BEN domain-containing genes as a new functional gene family in polydnaviruses. To determine a molecular interaction of BEN proteins, a BEN gene (CpBV-ORF3302) was expressed in Escherichia coli and purified. Pull-down experiments using recombinant BEN protein against cytoplasmic and nuclear extracts of P. xylostella larvae suggest that BEN may interact signal transduction and chromatin remodeling to alter host gene expression.

Keywords:*Cotesia plutellae, Plutella xylostella, BEN family, immune, nodulation, RNA interference, recombinant, expression, interaction.*

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O201M09

Physiology

O201

A viral histone H4 interacts with nucleosome components and alters target insect transcriptome revealed by an Illumina RNASeq

Rahul Pralhad Hepat[1], Yonggyun Kim[2]

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A viral histone H4 is encoded in a polydnavirus called Cotesia plutellae bracovirus (CpBV), which is symbiotic to an endoparasitoid wasp, C. plutellae. Compared to general histone H4, the viral H4 possesses an extra N-terminal tail containing 38 amino acid residues, which has been presumed to control host gene expression in an epigenetic mode. This study addressed its molecular interaction with host nucleosomes and its target genes to be altered in their transcription by an Illumina RNASeq. Reassociation of nucleosome components with a recombinant CpBV-H4 produced octamers containing each monomer of H4 and CpBV-H4 plus dimers of H2A, H2B, and H3, which were separated by a size-exclusion chromatography and analyzed by SDS-PAGE. Transient expression of CpBV-H4 in a model insect, Tribolium castaneum, was performed by microinjection of a recombinant expression vector and confirmed by RT-PCR and immunoblotting. Under this transient expression condition, total RNAs were extracted and read with an Illumina HiSeq 2000. Annotated transcripts were classified into different GO categories and compared with those of control insects injected with a non recombinant expression vector. Genes showing significant differences (P < 10-5) in corrected transcript levels were determined to be targets manipulated by expression of CpBV-H4. When the target genes were physically mapped, they were scattered on entire chromosomes of T. castaneum. Moreover, the target genes were not significantly different with a typical transcriptome at the developmental stage. These suggest that the viral histone H4 alters host gene expression by a direct molecular interaction with target insect nucleosomes without specificity.

Keywords:*Histone H4, polydnavirus, nucleosome, RNASeq, Illumina, Tribolium castaneum*

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O202M01

Physiology

O202

Utilization of microRNA mimics and inhibitors in pest control

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MicroRNAs (miRNAs) as ~21 nucleotide non-coding small RNAs have emerged as key factors in gene regulation involved in various biological processes. In this study, a larval-specific miRNA, har-miR-2002b, was identified from Helicoverpa armigera. For the first time, we explored the possibility of utilizing synthetic inhibitor and mimic of miRNA by oral feeding and confirmed that the inhibitor and mimic can successfully inhibit/oversupply the miRNA in H. armigera larvae. Bioassays by oral feeding indicated 70% reduction in fecundity and 40% larval mortality in the presence of har-miR-2002b mimic. In addition, a large proportion of those larvae that made it to the pupal stage were deformed and only few moths emerged from them. This implied an important role of har-miR-2002b in larval development and adult fecundity. A trypsin-like serine protease (Ha-TLP) was identified as a target of har-miR-2002b. In vivo and in vitro studies revealed that in the presence of the miRNA mimic Ha-TLP transcript levels were significantly down-regulated. In contrast, inhibition of har-miR-2002b led to significant up-regulation of Ha-TLP transcript levels. This is the first study which shows the potential of utilization of miRNA mimics and inhibitors in pest control by targeting specific insect genes.

Keywords:*Application of microRNA inhibitors, mimics in pest control*

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O202M02

Physiology

O202

Pigment synthesis pathway genes and their application to visible transgenic markers

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Ommochromes and melanins are major pigments involved in reddish and blackish coloration in insects. However, molecular mechanisms of the synthesis of these pigments are not fully understood. Here we report our recent findings on pigmentation mutants of silkworm Bombyx mori and their application to visible molecular markers. For the first topic, we will talk about the identification of the gene responsible for the egg and eye color mutant, red egg (re) by positional cloning, mutant analysis, and RNAi experiments. The eggs of re mutant display a pale orange color instead of normal dark coloration, and have been suggested to have a defect in the biosynthesis of the final ommochrome pigments. The gene responsible for the re mutant was a novel major facilitator superfamily transporter. Interestingly, the re gene homolog were found in all insect genomes sequenced at present, except for twelve sequenced Drosophila genomes, which seemed to correlate with the previous studies that have demonstrated that eye ommochrome composition is different from other insects in several Dipterans. For the second topic, we will report the development of a dominant visible marker for insect transgenesis using a gene responsible for a silkworm melanin synthesis mutant. This marker changed the color of newly hatched first-instar silkworm larvae from black to a distinctive light brown color. Suppression of black pigmentation could be observed throughout the larval stages and adult. The widespread distribution of melanin pigments suggests its potential to be applied to diverse insect taxa.

Keywords:*pigmentation, transgenesis, ommochrome, melanin, silkworm, Tribolium*

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O202M03

Physiology

O202

Transcriptome analysis of the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae)

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Bactrocera dorsalis is a widely spread polyphagous pest feeding on more than 250 fruits and vegetables. Currently, there is no ideal control program. To identify more molecular targets involved in reproduction and development in B. dorsalis and finally effective control on these pests, the transcriptome for the B. dorsalis from the whole body at different developmental stages was produced using 454 pyrosequencing. In a single run, 747,206 sequencing reads were obtained and assembled into 28,782 contigs and 169,966 singletons. Additionally, we identified a great number of genes involved in reproduction and development as well as genes that represented nearly all major conserved metazoan signal transduction pathways, such as insulin signal transduction. Furthermore, transcriptome changes during development were analyzed. A total of 2,977 differentially expressed genes (DEGs) were detected between larvae and pupae libraries, while there were 1,621 DEGs between adults and larvae, and 2,002 between adults and pupae. These DEGs were functionally annotated with KEGG pathway annotation and 9 of them were validated by qRT-PCR. Our data provide for the first time access to the genetic architecture of reproduction and development as well as major signal transduction pathways in the Tephritid fruit fly pests, allowing us to elucidate the molecular mechanisms underlying courtship, ovipositing, development and detailed analyses of the signal transduction pathways.

Keywords:*Bactrocera dorsalis, transcriptome*

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O202M04

Physiology

O202

Characterize the functions of candidate effectors in aphid by RNA interference

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Aphids are among the most devastating insects of plants. As a phloem-feeding insect, aphid punctures sieve tubes with stylet and uptakes phloem sap to ingest nutrients. From the onset of stylet penetration, aphid secretes two types of saliva into plant tissues: gelling saliva that forms a sheath surrounding stylet, and watery saliva that contributes to further penetration and ingestion. Similar to the interaction between pathogen and plant during invasion, some proteins and other chemical constituents in aphid's saliva may act as effectors which trigger or suppress plant's immune system. We focused on secretory proteins expressed in aphid's saliva to select effectors which are essential in feeding process, especially in the course of interaction between aphids and plants. To screen genes in salivary glands playing important roles in aphid feeding processes, double-stranded RNA (dsRNA) was delivered to aphids through artificial feeding. Considering Arabidopsis thaliana a model plant and powerful genome resource, Myzus persicae and Acythosiphon pisum were selected. We found that feeding dsRNA to the two aphid species, target gene transcript levels in treatment group ranged from 20% to 50% compared to control group. We also observed that knockdown some of the candidate genes can affect nutrients ingestion.

Keywords:*aphid, RNA interference, dsRNA, effector, saliva*

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O202M05

Physiology

O202

Social interactions and gene expressions involved in the soldier differentiation of a termite *Reticulitermes speratus*

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The complex social organization of termite colonies requires sophisticated regulatory mechanisms to adjust caste composition to the colony needs. In this aspect, the presence of soldiers is known to regulate soldier differentiation. Soldiers originate from workers. Their differentiation, which can be induced by juvenile hormone (JH) application, is inhibited in presence of soldiers. In our previous study, the presence of soldiers was shown to decrease JH titer in workers treated with JH III. However, what factors mediate the soldier presence and how JH titer is controlled are currently unknown. Here, we investigate these mechanisms using the lower termite Reticulitermes speratus. Firstly, we investigated the frequency of trophallaxis and the duration of grooming between workers and soldiers in the dishes treated with JH III. These behaviors are thought to be candidates transferring the soldier effects. As a result, we found that when they are treated with JH III, workers spend longer time grooming soldiers. Therefore, soldier effects seem to be transferred during grooming behavior. Secondly, we investigated the gene expression in workers reared in presence and absence of soldiers. Based on the qPCR of various genes involved in the JH pathway, our results showed that the expression of cytochrome P450 genes, namely, RsCYP4C46 and RsCYP15A1 decreased in workers reared with soldiers. These genes are thought to contribute to the JH synthesis and secretion, suggesting that the worker corpora allata are suppressed by the soldier presence, that in turn causes the rapid decrease of JH titer in workers.

Keywords:*termite, soldier differentiation, caste regulation, grooming, corpora allata*

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O202M06

Physiology

O202

Bactrocera dorsalis shows RNAi to orally feeding dsRNAs and bacteria expressing dsRNAs

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The oriental fruit fly, Bactrocera dorsalis, is a devastating agricultural pest in tropical and subtropical region, causing millions of loss to fruit crops such as citrus and guava. RNA interference is a powerful tool to inhibit target gene expression which makes this technology a useful method in IPM. Here we report that directly feeding dsRNA or engineered Escherichia coli strain HT115 expressing dsRNAs both caused RNAi effect in adult flies. Fragment of target genes were cloned into L4440. Then E.coli strain HT115 were transformed with recombinant vectors to express dsRNA in vitro. dsRNAs were designed targeting genes that encode ribosomal protein Rpl19, ATPase D, the fatty acid elongase Noa and GTPase Rab11. Adult flies were fed either by dsRNAs or engineered bacteria for continuous 7 days. Results show that both methods successfully cause RNAi in B.dorsalis. The maximum decrease of target gene expression vary from 35% to 100%. Tissue specific expression examination indicated that RNAi could be achieved in a tissue-specific way. Up-regulations of target genes were observed in the experiments using both methods. For example, in ds-rpl19 treated group, up-regulation occurred on day 2 and day 4 with each 3 times and 8 times compared to ds-egfp treated control group. This phenomenon could also be observed in ds-rab11 and all engineered bacterial treated group

Keywords:*Bactrocera dorsalis, feeding, RNAi, rpl19, rab11, v-type-ATPase-D, noa, up-regulation*

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O202M07

Physiology

O202

The Pax6/eyeless gene and the development of Collembolan eyes

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Collembola is a group of small, entognathous, and wingless basal hexapods. Some Collembolan species have 1+1 to 8+8 ommatidia, but some have no eye. Therefore, it's very interesting to study their eye polymorphism. Pax6/eyeless gene had been widely proved to be related with eye development across the animal kingdom. As a transcription factor, it contains two conserved domains (the Paired domain, PD and the Homeodomain, HD), a variable linker region and a C-terminal transactivation domain. In this study, we choosed two Collembolan species, the eyeless Folsomia candida and the eyed Ceratophysella denticulata (8+8 eyes) for comparison. First, the Pax6/eyeless genes of two species were cloned and sequenced. Their amino acids sequence of PD and HD showed high conservation, but other regions were highly divergent. Then, the transgenic Drosophila melanogaster with Pax6 genes from both species were explored to study the function. The intact Pax6 of the eyed Collembolan species can induce ectopic compound eyes on the legs and wings of adult fruitfly, although the induced eyes with reduced shapes and reduced ommatidia numbers. However, the intact Pax6 of the eyeless species can't. When the Pax6 C-terminal of the eyeless species was replaced with the corresponding protein region of the eyed species, the chimeric gene can induce ectopic eyes. On the contrary, the chimeric gene of eyed Pax6 gene with the C-terminal of eyeless species can't induce ectopic eyes in Drosophila. The preliminary results suggested that the C-terminal of Pax6 gene is crucial for the Collembolan eye polymorphism.

Keywords:*Collembolans, Pax6, eyeless gene, eye development*

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O202M08

Physiology

O202

Effects of plant growth promoting rhizobacteria (PGPR) on induction of plant volatiles and consequences for insect-plant interactions

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Plant growth-promoting rhizobacteria (PGPR) represent a wide range of root-colonizing bacteria whose application is often associated with increased rates of plant growth, suppression of soil pathogens, and the induction of systemic resistance. The effects of application of PGPR on induction of volatile organic compounds (VOCs) in treated plants and consequences for insect-plant interactions have gone largely unexamined. Studies were conducted to test the hypothesis that PGPR alters cotton plant VOCs with possible consequences for plant-insect and tritrophic interactions. Three PGPR strains were tested. Strains were applied as spore preparations and headspace volatiles were collected from PGPR-treated and untreated cotton plants four weeks after planting. Results showed quantitative and qualitative differences in headspace volatiles of PGPR treated versus untreated plants. Host acceptance tests with beet armyworm, Spodoptera exigua (Lepidoptera: Noctuidae) showed that females prefer to lay eggs on untreated cotton compared to PGPR-treated cotton plants. Four choice olfactometer behavior experiments with Microplitis croceipes (a hymenopteran parasitoid of cotton bollworm) revealed that PGPR-treated plants were highly attractive to the parasitoids. These results demonstrate that PGPR alters production of VOCs in cotton, and that herbivores and parasitoids are capable of exploiting these differences in VOCs.

Keywords:*PGPR, Rhizobacteria, Plant Volatiles, Insect-Plant Interactions, VOCs*

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O202M09

Physiology

O202

RNAi knockdown of DNA methylation affects gene splicing in the honey bee *Apis mellifera*

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Epigenetics is increasingly appreciated as a process to help explain how genomic sequences give rise to complex phenotypes. DNA methylation is known to regulate gene expression, but recent studies suggest it also may be involved in alternative splicing of transcripts. To investigate this phenomenon in honey bees, we developed a novel methodology for non-invasive high-throughput application of silencing RNAs in vivo in adult bees. Small interfering (si) RNA-nanoparticles complexes are administered to bees by nebulization and these complexes reach cells via the tracheal system. Using this method we treated bees with DNA methyl-transferase 3 (DNMT3) siRNAi and assessed results with RNAseq of abdominal tissue. Analysis of RNAseq data was performed via a novel computational program, TrueSight, that performs better than existing programs in providing quantitative measurements of alternative splicing. Analysis of control and DNMT3-RNAi transcriptomes revealed alternatively spliced isoforms for about 48% of the genes. DNMT3-RNAi caused statistically significant effects on 4 different types of splicing patterns: exon skipping, alternative start/terminal site, alternative 3'/5' site, and intron retention events. Gene Ontology analysis revealed that the set of genes showing DNMT3-RNAi caused differences in alternative splicing was enriched for phosphoprotein, nucleotide-binding, and ATP-binding genes. These results indicate that DNA methylation in honey bees plays a strong role in regulating alternative splicing of transcripts. Future studies will focus on the role of epigenetics in regulating honey bee phenotypic plasticity.

Keywords:*epigenetics, alternative splicing, apis mellifera, RNAi*

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O202M10

Physiology

O202

Comparative fat body transcriptome analysis of sunn pest in overwintering and summer populations

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Sunn pest is the main pest of wheat and barley in wide areas of Turkey and the Middle East. It overwinters at altitudes, usually under bushes of Artemisia, Kochia, Acantholimon. Its hibernation is a physiological requirement in terms of its survival. The most important modification between overwintering and feeding individuals is the difference in lipid metabolism. In most insects, stored lipid reserves are used during diapause in order to supply the energy demand. Fat body is rich in mRNA and it serves as an expression site of most genes. Roche 454 pyrosequencing has become a method of choice for generating transcriptome data from non-model organisms. In this study, we have generated two different cDNA libraries from the fat bodies of feeding adults in summer and dormant adults in winter in order to characterize the genes controlling lipid storage mechanism which is a necessity in sunn pest's biological cycle. We obtained more than 200.000 high quality sequencing reads from a 454 GS FLX Titanium pyrosequencer (mean length: approximately 400 base pairs). We analyzed the putative fat body transcriptome based on homology to other gene products with known functions available in the public domain. We found genes that showed differential overall expression by comparison of two transcriptomes. Our sequencing and annotation framework will provide a simple and effective way to achieve high-throughput gene discovery for organisms lacking a sequenced genome. Furthermore the database revealed from this study will also provide a valuable resource for developing specific pest management strategies.

Keywords:*Sunn pest, fatbody, transcriptome analysis, insect diapause*

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O203TU01

Physiology

O203

Is older stronger? Correlates of ester metabolism with task-related polyethism in *Apis mellifera*

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Esterases have been considered among the main detoxification enzyme groups in insects. Due to task allocation in honey bee social development, we hypothesized that polyethism would be accompanied by variation in enzyme activities in individuals according to caste and task. The metabolism of seven ester compounds in honey bees was assayed in vitro. Our study shows that the midguts from workers in different stages (newly emerged worker, nurse bee and pollen forager) and from drones are all capable of detoxifying aliphatic esters [octyl butyrate(OB), octyl acetate(OA), hexyl butyrate(HB) and butyl butyrate(BB)] and monoterpenes [myrtenyl acetate(MA), thymyl acetate (TA) and perillyl acetate(PA)]. However, nurse bees and pollen foragers have higher esterase-mediated metabolic rates than newly emerged workers and drones for OA, HB, BB and MA. Because nurse bees are responsible for feeding the grubs, their elevated rates of ester metabolism may be related to the requirement of processing the raw food, such as pollen, which is not possessed by newly emerged workers and drones. Also, the esterase system may need time to develop in newly emerged workers. Moreover, older bees usually produced a lower amount of 1-octanol, a product of OA or OB metabolism, but they consume substrates at higher rates than younger bees. Since 1-octanol is one of the components of the honey bee alarm pheromone, young bees, which would be unlikely to participate in a major way in colony defense, may not have the capacity to metabolize alarm pheromone.

Keywords:*Apis mellifera, esterase, division of labour*

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O203TU02

Physiology

O203

Research progress of target and detoxification enzymes related to resistance of *Bactrocera dorsalis*

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The oriental fruit fly, Bactrocera dorsalis, is a major pest causing severe damage to agriculture worldwide, and developed insecticides resistance rapidly. We have conducted the transcriptome analysis of B. dorsalis, obteined a huge number of gene sequences encoding insecticide targets and detoxification enzymes. Based on the data, a series experiments were carried out to investigate specific gene functions related to resistance. Biochemical and molecular characteristic analysis of purified AChE from four field populations and different developmental stages proved that decrease of susceptibility of B. dorsalis was mainly caused by the decrease of AChE activity. It was in accordance with previous study that the alterations of AChE were correlated with resistance to organophosphate insecticide. The full cDNA sequences of other important target genes were sequenced including a VGSC gene (6446 bp), and four nAChR genes. PCR-RFLP was used to identify whether point mutations related to resistance existed. Eighteen full sequences of GST genes were isolated. Their expression profile among developmental stages, different tissues, and insecticides induced strains were detected. The results suggested that GSTs were involved in detoxification of abamectin and beta-cypermethrin. Fifty-one partial sequences of P450 genes were identified in transcriptome data, and 15 of them were fully sequenced. With the progress of cloning, a solid basis would be made for the identification of their diversity and detoxification of insecticides. Now, accomplishment of genome project of B. dorsalis provides an opportunity to get a comprehensively understanding of its resistance mechanism, and will improve the resistance management of this pest.

Keywords:*Bactrocera dorsalis, resistance, transcriptome, AChE, GST, P450*

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O203TU03

Physiology

O203

The effects of Xanthotoxin on some biochemical parameters of *Galleria mellonella* (Lepidoptera: Pyralidae)

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The effects of a plant allelochemical, xanthotoxin (XA), on important oxidative stress indicators, lipid peroxidation product, malondialdehyde (MDA) and protein oxidation products, protein karbonyl (PCO) contents and a detoxification enzyme glutathione-S-transferase (GST) activity were investigated in the adults of the greater wax moth Galleria mellonella (L.). The insect was reared from first-instar larvae on an artificial diets containing XA at 0.001, 0.005 or 0.1% to adult stage in the laboratory condition. The lowest concentration of XA (0.001%) significantly increased MDA content from 0.005 ± 0.0005 to 0.028 ± 0.001 nmol/mg protein by approximately 6-fold while it had no significant effect on protein carbonyl content and GST activity. XA at high concentrations (0.005 and 0.1%) significantly increased MDA (3-fold) and protein carbonyl (2-fold) contents while they significantly resulted in decreased GST activity. The highest dietary XA concentration significantly decreased GST activity from 0.276 ± 0.025 to 0.159 ± 0.005 µmol/mg protein/min. Two of the markers indicated that significantly higher levels of oxidative stress were produced in the whole body of adult reared on diets supplemented with XA. This oxidative stress was associated with elicited detoxification responses by markedly decreasing GST activities in adults. We infer from these findings that XA-induced oxidative stress exerted strong inhibitory effect on the activity of the wax moth detoxification enzyme leading to decreased adult fitness.

Keywords:*Galleria mellonella, oxidative stress, xanthotoxin, GST, protein carbonyl*

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O203TU04

Physiology

O203

Effects of ethacrynic acid on characterizations of GSTs from *Liposcelis bostrychophila*

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Glutathione S-transferases (GSTs) are a large family of multifunctional enzymes found ubiquitously in aerobic organisms. In order to clarify the effects of ethacrynic acid (EA) on the biochemical and toxicological characterizations of GSTs from Liposcelis bostrychophila (Psocoptera: Liposcelididae), GSTs from the psocid rearing on the diet with 0.5% EA or 3% EA for 30 days was purified by glutathione-agarose affinity chromatography, and characterized subsequently by their Michaelis-Menten kinetics toward the artificial substrates 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH). The bioassay results revealed that the psocids on the diets including different contents of EA both exhibited more sensitivity to deltamethrin compared to the control, the psocids on the normal diet. The specific activity of the affinity of purified GST toward CDNB was highest in the control, 1.87- and 10.00- fold higher than the counterparts of the psocids on 0.5% and 3% EA diets, respectively. GSTs of psocids after EA treatment exhibited lower maximal velocity values for both the substrates above than that of control. However, the apparent Michaelis-Menten constants differed a lot with higher values for the substrated GSH while lower for CDNB, revealing EA treatment induced GSTs to express a significant lower affinity to the GSH. Inhibition kinetics showed that all test compounds (EA, diethyl maleate, and deltamethrin) possessed significant inhibitory effects on GSTs and the enzyme indicated less sensitivity to the compounds after continous EA treatment.

Keywords:*ethacrynic acid, GSTs, purification, psocids, xenobiotic compounds*

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O203TU05

Physiology

O203

Stress response and metabolomic defects in apoptosis-deficient mutant in *Drosophila melanogaster*

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Multicellular organisms have evolved machineries for tissue remodeling to get rid of damages by apoptosis resulted from environmental stresses. Apoptosis is a programmed cell death, which is executed by evolutionary-conserved cysteine proteinases, caspases. Because tissue remodeling is essential for any organisms to maintain homeostasis, dysregulation of apoptosis leads to major pathologies such as cancer. There is increasing evidence that apoptotic machinery is correlated with inflammation, proliferation, and metabolic homeostasis, which are also altered in cancer cells. However, it is not known so far what occurred practically in vivo if apoptosis is defective. In this study, we focused on stress response and metabolic alteration in Drosophila apaf-1 (dark/hac-1/dpf-1) hypomorphic mutant, in which caspase activation and subsequent apoptosis were severely impaired. We found that dpf-1 mutant was sensitive to starvation- and oxidative-stress. Starvation susceptibility was resulted from "wasting" phenotype as revealing their shortage of energy storage. Next, we conducted metabolomic analysis of hemolymph in dpf-1 mutant using capillary electrophoresis-mass spectrometry (CE-MS) system to investigate the physiological consequences of apoptosis defects. Several metabolites were increased in dpf-1 mutant, which was likely due to the alteration of expression level of putative metabolic enzymes in fat body at least for one of such metabolites. We have succeeded in modulating the level of the metabolite through genetic manipulation of metabolic enzymes and tested whether alteration of metabolite affected stress sensitivity. We will present and discuss the physiological role of apoptosis based on investigation of metabolic alteration in dpf-1 mutant.

Keywords:*Drosophila, apoptosis, metabolomics, stress response, cancer*

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O203TU06

Physiology

O203

Winter climate change: Elevated adult temperature results in epigenetic loss of cold tolerance in overwintering larvae of the blowfly, *Calliphora vicina*

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As ectotherms, insects are particularly vulnerable to global warming. Current rates of warming have altered the phenology and distribution of numerous insect species, while virtually nothing is known about how global warming has affected the ability of insects to successfully overwinter. This is surprising, as winter periods with limited nutrients and periods of extreme cold are the most challenging for temperate insects. Virtually all temperate insects utilise diapause as their overwintering strategy. Typically this is initiated by change in an environmental cue. For example in Calliphora vicina adult females detect shortening photoperiod, signalling the advent of winter, while their progeny overwinter as diapausing larvae. Global warming threatens to expose adults to warmer late-autumn temperatures, while diapausing larvae will still experience periods of severe mid-winter cold. The effect this will have on the epigenetic inheritance of cold tolerance was investigated by culturing adults at 15 and 20ºC and assessing cold tolerance in their progeny. It was found that adults cultured at higher temperatures produced diapausing progeny with a less intense diapause, reduced supercooling ability and increased mortality following exposure to -4 and -8ºC. This provides evidence that warmer late-autumn temperatures may result in reduced cold tolerance and therefore elevated mortality in the diapausing life-stage. Interestingly the effect of adult thermal history was not detected in cold tolerance of non-diapause larvae, indicating the cross-generational programming of diapause is closely linked to cold tolerance. Underlying mechanisms are still to be identified but may involve altered lipid-membrane composition or altered expression of heat-shock proteins.

Keywords:*Diapause, Climate change*

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O203TU07

Physiology

O203

Apoptosis and autophagy in the midgut of the American cockroach, *Periplaneta americana* under short-term and long-term starvation

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Short-term starvation, of 2 weeks, does not affect mortality in Periplaneta americana. However long-term starvation, of 4 weeks, reduced the number by half and affected the midgut structure, inducing many small condensed nuclei in the nidi, nests of stem cells and newborn cells. Apoptosis often exhibits nuclear condensation with chromatin concentration. Autophagy, a self-digestion, may be induced in the midgut of the starved cockroaches but it is unclear whether short or long-term starvation stimulates autophagy. This study investigates effects of short or long-term starvation on apoptosis and autophagy in the midgut of P. americana, histochemically, ultrastructurally, and at molecular biology level. During short-term starvation, cell death began to increase in the columnar cells. However long-term starvation reduced the cell population and showed a significant increase in cell death in the nidi. The cell death in the nidi was apoptosis because of the active-caspase 3-like immuno-reactivity and chromatin condensation under electron microscopic observation. Translation and activation of specific autophagic marker, ATG8 was stimulated drastically in long-term starvation. These results suggest that short-term starvation gradually increased the cell death in the midgut epithelium, whereas long-term starvation induced apoptosis in the nidi and autophagy in the midgut. P. americana probably shift the defense mechanism according to the progression of starvation from optimistic conditions to emergency situations.

Keywords:*Apoptosis, autophagy, cockroach, midgut, starvation*

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O203TU08

Physiology

O203

Flight performance of bumblebees under Mars' micro gravity (0.38G)

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Flight performance of bumblebees (Bombus ignitus) under micro gravity was studied using parabolic flight of Jet plane (MU-300), to see if the bumblebees can fly under zero or micro gravity (such as in Int. Space Station), or under the Mars' gravity (=0.38G). Various strength of micro gravity condition was made by parabolic flights (ca 30s x 10 to 12 times per flight) and the bees in flight chambers were triggered to fly by the onset of illumination with UV-LED. The number of bees kept in a chamber was ca 25 in a big one (30 x 30 x 30 cm), 3 in a small one (15 x 20 x 5cm in depth). Each chamber (one large and 3 small ones) was monitored by different type of video recorders including high speed of 250 flames per second. The bees could take off and began to fly but could not fly normally in 0G. They fly with high speed after few strokes of wings and hit the chamber wall. But under 0.38G, they tried to keep balance in hovering-like motion during their flight. We could not confirm whether they can fly in long distance or long duration, because the duration of controlled micro gravity was 30 seconds in maximum in this experiment. The evidence suggests that the bumble bees can be used in future as possible pollinator in the crop production in Mars.

Keywords:*Bumblebee, micro gravity, flight, Mars*

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O203TU09

Physiology

O203

Oxidative stress is a major factor for the induction of anhydrobiosis in the desiccation-tolerant midge, *Polypedilum vanderplanki.*

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In the semiarid regions of Africa, larvae of the sleeping chironomid (Polypedilum vanderplanki) are able to survive in a completely desiccated state without any metabolic activity throughout the dry season, which may last for 8 months. This phenomenon is known as anhydrobiosis. Upon rehydration, dry larvae can recover normal activity in less than one hour. In order to identify genes involved in the anhydrobiosis of P. vanderplanki, EST libraries were constructed from normally active, desiccating and anhydrobiotic larvae. EST and microarray analyses showed the up-regulation of desiccation responsive genes, such as antioxidants, Late Embryogenesis Abundant (LEA) proteins, HSPs, transporters and trehalose synthesizing enzymes, during the induction of anhydrobiosis. Oxidative stress was apparently a major damaging factor during anhydrobiosis and in spite of the up-regulation of many antioxidant genes, rehydrating larvae experienced severe oxidative damage on proteins, DNA and biological membranes. Microarray analysis of larvae treated with the oxidative reagent Paraquat (PQ) showed that in addition to the classical antioxidant response, general desiccation-related gene expression was also observed after treatment, suggesting that apart from their harmful effect, reactive oxygen species (ROS) can also act as important signaling molecules. Functional analyses using PQ and an antioxidant confirmed that ROS were directly involved in the regulation of gene expression during the induction of anhydrobiosis. We propose that the oxidative stress response pathway has been co-opted for the induction of anhydrobiosis in P. vanderplanki and further functional analyses will focus on the genes involved in ROS sensing.

Keywords:*Transcriptomics, Diptera*

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O203TU10

Physiology

O203

Frost expression is not essential for recovery from chill coma, acute cold tolerance, and rapid-cold hardening in *Drosophila melanogaster*

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Temperature affects many physiological processes in insects and surviving winter sub-zero temperatures is critical for many insects in temperate and polar zones. Cold tolerance in insects is enhanced not only by long term acclimation to low temperature, but by short periods of non-lethal cold exposure, rapid cold-hardening (RCH). Although some previous studies have reported physiological processes of the RCH response, the mechanisms underlying RCH are still not clear. Frost is up-regulated during recovery from cold exposure in D. melanogaster. Previous work showed that knockdown of Frost increases chill-coma recovery time, but the role of Frost in acute cold tolerance and RCH remains unknown. To clarify the contribution of Frost to cold tolerance, we used several D. melanogaster lines with Frost expression knocked-down with the GAL4/UAS system and obtained lines with varying Frost expression levels. Chill-coma recovery time, survival rate after acute cold exposure and RCH were measured in each line. There was no correlation between gene expression levels and three different types of cold tolerance, suggesting that Frost is not essential for those cold tolerance phenotypes.

Keywords:*Frost, RNA interference, chill coma recovery, rapid cold hardening*

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O203TU11

Physiology

O203

Influence of hexane extract of *Ocimum sanctum* on longevity and reproduction of *Dysdercus koenigii*

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Impact of Hexane extract of Ocimum sanctum, was evaluated on longevity and reproduction of Dysdercus koenigii. Sexually mature adults and 5th instars were treated with 10%, 5%, 2.5% and 1.25% of hexane extract of Ocimum leaves. When adults were treated with 10% extract mortality was 100% and they died on an average within 4 days. 5% of hexane extract was more toxic to males than the females. Also the longevity of the treated males was lesser than the treated females. A concentration of 2.5% and 1.25% did not affect longevity of the insect significantly. The hexane extract of Ocimum has profound effect on the mating behavior of Dysdercus. The adults when treated with 5%, 2.5% and 1.25% of hexane extract exhibited disruption of courtship behavior. As a result mounting attempts and successful mating rate was reduced. This is in contrast to normal adults which mate immediately after releasing in to the jars. The extract also altered oviposition behavior of the females. The treated females laid lesser number of egg batches, number of eggs in each egg batch and total number of eggs laid in their entire lifespan. The eggs laid by treated females had lower hatching success. When fifth instars were treated with Ocimum leave extract their moulting success was decreased. They retained nymphal characters or turned in to adultoids after moulting. There was a direct correlation in concentration of extract and the deformities observed in the fifth instar nymphs The supranumeric instars and adultoids failed to mate and reproduce.

Keywords:*Dysdercus,Ocimum, Longevity, Reproduction, Mating behaviour*

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O203TU12

Physiology

O203

Determination of catecholamines and aminoacids from venom reservoir extract of honeybee (*Apis*) species

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Catecholamines system plays a variety of roles in animals, as neurotransmitters, neuromodulators, hormones, paracrine and autocrine factors. Neumerous venoms of widely different animals have evolved to manipulate these systems for both defensive and offensive purposes of the venomous animals. Catecholamines are sympathomimetic and comprises of catechol group derived from the amino acid thyrosine. Amino acids are essential building blocks of proteins and peptides. Physiological and anatomical evidence suggests that certain amino acids act as neurotransmitters of the major excitatory or inhibitory commands in the central nervous system and also take part in intermediary metabolism. The catecholamines viz., epinephrine (E), norepinephrine (NE), dopamine (D), 3,4 dihydroxyphenyl acetic acid (DOPAC), 5- hydroxyindole-3-acetic-acid (5-HIAA), 5- Hydroxytryptamine (5HT) and 4-hydroxy-3-methoxy-phenylacetic acid (HVA) were quantified from venom gland with reservoir extract of Apis cerana, Apis dorsata and Apis florea using Liquid chromatography with electrochemical detection (ECD) method. The quantitative results showed species dependent variation. The other two vital bioactive components examined namely DOPAC and HVA were absent in the three examined honey bee species and 8 amino acids (Aspartate, Glutamate, Serine, Alanine, Glysine, Phenyl ethanolamine, GABA and Tyrosine) were analysed to compare their presence in venom of three Apis species viz., A. dorsata, A. cerana, A. florea. All the 8 amino acids assayed were present in all the three species but the quantity of each amino acid differed in the venoms from different species of honeybees.

Keywords:*Catecholamines, Apis species, Liquid chromatography, Electrochemical detection, venom resoervior*

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O203TU13

Physiology

O203

Oxygen limitation of thermal tolerance in aquatic insects

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Thermal limits may arise through a mismatch between oxygen supply and demand in a range of animal taxa. This oxygen limitation hypothesis is supported by data from a range of marine organisms butit is unclear whether oxygen limitation determines thermal extremes in tracheates including insects. We demonstrate that thermal limits do indeed respond to external oxygen supply in the aquatic life stages of Plecoptera, suggesting that the critical thermal limits of such aquatic larvae are set by oxygen limitation. These findings have implications for our understanding of the limits to insect body size and how these are influenced by atmospheric oxygen levels, and indeed a larval view of Palaeozoic insect gigantism may be very informative, explaining the predominance of aquatic life stages amongst giant insects.

Keywords:*thermal limits, oxygen limitation, body size, Palaeozoic gigantism*

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O203TU14

Physiology

O203

Changes in energy metabolites as well as body color phenotypes for starvation and desiccation resistance in montane populations of *D. melanogaster*

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Storage of energy metabolites have been investigated in different sets of laboratory selected desiccation or starvation resistant lines but few studies have examined such changes in wild-caught populations of D. melanogaster. In contrast to parallel selection of desiccation and starvation tolerance under laboratory selection experiments, opposite clines were observed in wild populations of D. melanogaster. We tested whether there is a trade-off for storage as well as actual utilization of energy metabolites in D. melanogaster altitudinal populations. For desiccation resistance, darker flies store more body water content and endure greater loss of water as compared to lighter flies. Based on within population analysis, we found evidence for coadapted phenotypes i.e. darker flies store and actually utilize more carbohydrates to confer greater desiccation resistance. In contrast, higher starvation resistance in lighter flies is associated with storage and actual utilization of greater lipid amount. However, darker and lighter flies did not vary in the rate of utilization of carbohydrates under desiccation stress; and of lipids under starvation stress. Thus, we did not find support for the hypothesis that a lower rate of utilization of energy metabolites may contribute to greater stress resistance. Further, for increased desiccation resistance of darker flies, about two-third of total energy budget is provided by carbohydrates. By contrast, lighter flies derive about 66% of total energy content from lipids which sustain higher starvation tolerance. Our results support evolutionary trade-off for storage as well as utilization of energy metabolites for desiccation versus starvation resistance in D. melanogaster.

Keywords:*Body color phenotypes, Energy metabolites, Starvation and desiccation resistance, D. melanogaster*

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O204TH01

Physiology

O204

Discovery of woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae) biotypes in Australia

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The woolly apple aphid (WAA), Eriosoma lanigerum Hausmann (Hemiptera: Aphididae), is a worldwide pest of apple orchards that has become widespread in Australia. Several populations of this pest insect that are geographically distant may have evolved into distinct biotypes having different parameters of fitness (e.g. fecundity) on the same food source. In this study, we investigated the performance of four different lines of WAA (ALB, STA, BAT, and YAR) originating from four different locations of Victoria and New South Wales. Each line was grown from a single apterous female. In a glasshouse trial, we monitored the percentage of nymphs that established after 7 days, the percentage of nymphs that successfully reach the adult stage, the nymph developmental time on whole apple trees (cv. Granny Smith). We also measured adult weight and female fecundity. Our results showed that the four lines of WAA examined in the scope of this study can exhibit significant differences either in establishment success or in fecundity. Female fecundity, adult weight and developmental time were significantly higher in STA relative to ALB, while there was no significant difference of establishment success between these two biotypes. However, establishment of YAR was the lowest (42%) compared to biotype STA (64%). Our results demonstrate the existence of at least three different biotypes of WAA in Australia. Antibiosis and antixenosis could explain respectively the differences in aphid establishment and in fecundity of WAA biotypes. The discovery of WAA biotypes can have major implications for the management of this pest.

Keywords:*apple, aphid, biotype*

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O204TH02

Physiology

O204

Aphid alarm pheromone: Recent advances on biosynthesis and functions

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Aphids are among the most abundant and destructive insect pests of agriculture, particularly in temperate regions, causing direct damage to arable and horticultural crops as well as serving as vectors for many important plant diseases. As with most other insect interactions, the signals that mediate conspecific interactions among aphids are primarily chemical; and the most prominent and well-studied interactions among aphids are those mediated by "alarm" pheromones. The aphid alarm pheromone - of which the sesquiterpene (E)-β-farnesene is the key (or only) component in most species - plays important roles in mediating interactions among individuals as well as multitrophic interactions among plants, aphids, and aphid natural enemies. The alarm pheromones of several aphid species have been isolated and identified and, though many important questions remain to be answered, a large body of research has addressed various aspects of the biology, physiology, and ecology of aphid alarm pheromones (as recently reviewed by Vandermoten et al. in Insect Biochemistry and Molecular Biology 42 (2012) 155-163). Especially, we contributed to recent advances in the understanding of aphid alarm pheromone biosynthesis by the characterization of aphid prenyltransferases producing both geranyl diphosphate and farnesyl diphosphate which are the precursors of mono- and sesquiterpenes, respectively. The information gained from a deeper understanding of the biosynthesis and regulation of aphid alarm pheromone as well as the chemical ecology of aphid-natural enemy interaction will enhance our understanding of the biology and ecology of aphids and may facilitate the design of novel control strategies.

Keywords:*Aphid, Alarm pheromone, (E)-β-farnesene, Tritrophic interactions, Pheromone biosynthesis*

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O204TH03

Physiology

O204

Analysis of the yolk proteins expression in the male reproductive system of *Drosophila melanogaster*

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Egg storage proteins (YP's) are produced in the female fat body and ovary and participate in egg maturation. This class of proteins is not expressed in males under physiological conditions. In this study we demonstrate that the male reproductive system of Drosophila melanogaster most likely produces yolk proteins under normal conditions. Using 2-D electrophpresis and protein sequencing, we obtained sequences that are homologous to yolk proteins 1 and 2 in male reproductive system. We examined the presence of these proteins in the testes and tissues of the male reproductive tract using an antibody against YP's in immunocytochemical and Western blot analysis. Our results show that the YPs are present in testes, luminal fluid and accessory glands. In addition, they are also present on the surface of sperm from the seminal vesicles. We prove that their level changes in a rhythmic manner - this rhythm persists in light/dark cycles. Our findings suggest that yolk proteins are most probably expressed in cyst cells in the testes and accessory glands, transported with the fluid to the seminal vesicles and bind to sperm in this organ. Supported by NCN Grant No. N N303 595639 to P.B.

Keywords:*yolk proteins, sperm, rhythm, reproduction*

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O204TH04

Physiology

O204

Differential gene expression between fat body of male and female in oriental fruit fly, *Bactrocera dorsalis*

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The fat body plays many roles in insects. Most hemolymph proteins are synthesized in the fat body. The gene expression differences of fat body between 8-, 9-, and 10-day old male and female Bactrocera dorsalis were performed using the suppression subtractive hybridization method. 993 male specific clones were partially sequenced and searched using BLAST. About 21 % of these clones showed homology with detoxification enzymes including cytochrome P450s (Cyps) and glutathione S-transferases (GSTs). NADH dehydrogenases and storage protein receptor (SPR) which distributed to energy metabolism constituted about 16 % of these clones. About 9 % of these clones were antimicrobial peptides (AMPs). Cyp, GST, NADH dehydrogenase, SPR and AMP were also probed for microarray assay. Both microarray assay and real-time quantitative PCR showed that the five transcripts were expressed at higher levels in fat body of male than that of female. These results suggested while yolk protein synthesis is the main function of fat body in female, Cyp, GST, NADH dehydrogenase, SPR and AMP play important roles in male for its survival.

Keywords:*Bactrocera dorsalis, fat body, differential gene expression, suppression subtractive hybridization, microarray assay, real-time quantitative PCR*

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O204TH05

Physiology

O204

Molecular cloning, expression patterns and RNAi analysis of vitellogenin receptor from the brown planthopper, *Nilaparvata lugens* (Stal)

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The brown planthopper (BPH), Nilaparvata lugens, is the most destructive pest of rice throughout Asia. The vitellogenin receptor (VgR) mediates the uptake of vitellogenin (Vg) into developing oocytes during egg formation in insects. In this study, the cDNA of N. lugens VgR (NlVgR) was cloned and sequenced. The full-length cDNA of NlVgR is 6174 bp with an open reading frame of 5796 bp, encoding a protein of 1931 amino acids. Quantitative real-time PCR (RT-qPCR) and Reverse Transcription- PCR (RT-PCR) analyses revealed that NlVgR mRNA was specifically expressed in ovarian tissues. The developmental expression profiles showed that NlVgR mRNA was first transcripted in 1st day of brachypterous adults and reached the highest level on 9th day. Western blot analysis detected an ovary-specific VgR protein with a MW of ~215 kDa and protein temporal profiles were the same as mRNA expression patterns. RNA interference (RNAi) showed that 1st day of brachypterous adults injected with 100 or 250 ng VgR-dsRNA disrupted egg formation and significantly decreased the number of offspring. Western blot revealed that vitellogenin (Vg) concentration in haemolymph was increased and Vg content in ovary was decreased. NlVg mRNA expression level and total Vg protein content of whole body were not changed. These results show that VgR mediate the incorporation of Vg into oocytes during egg development and plays a crucial role in reproductive process of the BPH .

Keywords:*Nilaparvata lugens, vitellogenin receptor, RNA interference, double-stranded RNA, microinjection.*

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O204TH06

Physiology

O204

Fenoxycarb induced disturbance of testes development, apoptosis of germ cells and sperm release in moths

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Application of fenoxycarb, a potent juvenile hormone analogue (JHA) belonging to insect growth regulators (IGR), induces different morphological, physiological and behavioral changes during development and metamorphosis of insects. The administration of fenoxycarb to Galleria mellonella larvae blocks metamorphosis, causing a significant elongation of larval development and leading to supernumerary larval instars. In many studies authors indicate the strong influence of fenoxycarb on the reproduction in females. In this study we present the effect of fenoxycarb on male reproductive system in Galleria mellonella larvae. Effect of JHA on the structure of testes and spermatogenesis was analyzed for 28 days since fenoxycarb administration, until larval-pupal intermedians or deformed pupae appeared. Characteristic feature of male reproductive system is fusion of paired testes into one organ, present in adult males. In insects treated with the juvenoid, testicular fusion does not occur, and a portion of the spermatocytes is eliminated through apoptosis. The spermatocytes, which are developmentally advanced, enter the stage preceding the first meiotic metaphase and then degenerate in the process restricted to several characteristic events. We observed nuclear envelope disruption, an aberrant nucleus containing heterochromatic bodies seen as an electron-dense material, and finally the lysis of whole cells. The histochemical study demonstrates that all of these processes are of an apoptotic character. Another aspect of our study was the effects of fenoxycarb on sperm release in adult males of two lepidopterans, mentioned above Galleria mellonella and Spodoptera littoralis, model organism for circadian research. Supported by NCN grant No. N N303 803340 to MAP.

Keywords:*juvenile hormone analogue, fenoxycarb, spermatogenesis, rhythms*

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O204TH07

Physiology

O204

Studies on reproductive behaviour of susceptible and insecticide-resistant *Plutella xylostella* (Linnaeus)

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Diamondback moth (DBM), Plutella xylostella (Lepidoptera: Plutellidae) is cosmopolitan and most noxious pest of cruciferous crops and has developed resistance to more than 46 insecticides including those with novel modes of action. One of the mechanisms of insecticide resistance is behavioural mechanism and was studied w.r.t. reproductive behaviour of P. xylostella. Influence of the test-insecticides was observed on various reproductive parameters of P. xylostella by allowing the freshly emerged adults to mate in four different combinations i.e. resistant male mated with resistant female (RM×RF), resistant male mated with susceptible female (RM×SF), susceptible male mated with resistant female (SM×RF) and susceptible male mated with susceptible female (SM×SF).No significant difference was observed among the various combinations as regards pre-oviposition and post-oviposition period. Oviposition period, however differed significantly for RM×RF i.e. 8.33 days. Hence, the maximum fecundity was recorded for RM×RF i.e. 185.0 and was significantly more than all the other three combinations. It is noteworthy that highly appreciable reduction of the egg-size (0.023 mm3) was recorded in the combination RM×RF. An egg-size of 0.035 mm3 was recorded for combination SM×SF which was significantly more than the other combinations. Reproductive effort (fecundity × egg-size) was compared for all the four combinations and no significant difference was noted. Hatchability of the eggs from the combination SM×SF (79.28 %) was significantly more than that RM×RF combination where it was 74.10 percent.

Keywords:*Egg-size, fecundity, hatchability, insecticide-resistance, oviposition, Plutella xylostella*

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O204TH08

Physiology

O204

Effects of hen egg white proteins on reproductive performance of red flour beetle, *Tribolium castaneum* (Coleoptera:Tenebrionidae)

Megha Kansal[1], Geetu Choudhary[2], Ranjit Kaur Parshad[3]

[1]Punjab Agricultural University, India, [2]Punjab Agricultural University, India, [3]Punjab Agricultural University, India

Tribolium castaneum (Herbst), being among the most adaptive and problematic pest of stored-products. Hen egg white comprises growth inhibiting dietary proteins against many Coleopteran species and moths. Therefore, the present work describes the evaluation of insecticidal activity of egg white proteins on the reproductive parameters of Tribolium castaneum. Protein samples obtained through salt fractionation (PPT-I and PPT-II) including whole egg white (WEW) were lyophilized. Different concentrations (2%, 3%, 4%, 5%, 10%) of each of the protein samples were tested by adding them to wheat flour and milk powder (75:25 w/w). The fecundity rate, reproductive effort of the beetles were significantly ($p<0.05$) lower in 3%, 4%, 5% and 10% of both PPT-I and PPT-II than that of the control group after 30 days of treatment. Marked abnormalities were observed in appearance and dimensions of ovarioles and eggs which indicated that the egg protein samples caused considerable effect on the process of oogenesis. Scanning electron microscopy studies revealed changes in the structure of epithelial sheath cells of ovarioles probably caused due to resorption of ova in the beetles fed on PPT-II proteins. Higher resolution of the egg surface showed the presence of wrinkles or shrivelled appearance of the chorionic layer in PPT-I and PPT-II treated eggs and these deep folds were more conspicuous in the beetles fed on PPT-II proteins. The findings reveal that hen egg white proteins could reduce the general fitness of an insect and thus the reproductive capacity.

Keywords:*Eggs, Egg white Proteins, Ovarioles, Oogenesis, Reproductive performance, Tribolium castaneum*

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O205TH01

Physiology

O205

Male calling and the reflex arc In *Leptophyes punctatissima*

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The flightless bushcricket Leptophyes punctatissima has a widespread distribution in Europe. Males and females communicate using ultrasound and the speed of communication is so fast that the neural circuit involved in the female reply to the male call is a reflex arc. The height at which males call, the sites they choose and the auditory and ecological problems that these pose, are part of a long-term study of acoustics and mate choice in this unusual species. Observations on adult mating behaviour were made at night, adults being located using directional ultrasound detectors. The approximate position of singing individuals was determined using paired detectors linked to an oscilloscope and the height of the singing male above the ground was calculated. Males were recorded at a range of heights between ground level and 14 m, a substantial height for a small flightless bush cricket. The choice of singing sites, the use of very short signals and the speed of reply of the females will be discussed in the context of the mating behaviour of this species together with the underlying neural pathway in the female.

Keywords:*Ultrasound, bush cricket, relex arc, time window*

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O205TH02

Physiology

O205

Olfactory and gustatory receptor genes in *Trissolcus basalis*

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Trissolcus basalis (Hymenoptera: Platygastridae) is an important egg parasitoid in the biological control of stinkbugs, including Nezara viridula (Pentatomidae). Gustatory (Gr) and odorant (Or) receptors proteins are components of the insect chemosensory system that bind to odor molecules and induce neuronal signalling. The structure of these proteins is thought to determine their ligand-binding properties, conferring the ability to detect kairomones and locate host eggs. These genes are subject to extensice duplication and loss, and likely play an important evolutionary role in shifts of host choice and speciation. Here we present annotation of gustatory and odorant receptor genes in T. basalis as the first step in a project to understand how gene evolution affects host choice and speciation within the genus Trissolcus. We compare our results with the arrays of Or and Gr genes in Apis mellifera, Nasonia vitripennis (Hymenoptera.: Pteromalidae), and 4 species of ants.

Keywords:*Trissolcus, olfactory receptor, gustatory receptor, host selection*

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O205TH03

Physiology

O205

Olfactory conditioning in the German cockroach

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The German cockroach is an important pest in association with humans world-wide. Various food odors were used as bait attractants to control them. It is believed that the cockroaches locate food by remembering learned general odor, so we could exploit this nature for an efficient control of the pest. In this study, we investigated the capability of learning and memory in the German cockroach. Firstly we carried out a differential type conditioning by presenting (-)-menthol odor with sucrose solution reward, followed by vanillin odor with saline punishment. Cockroaches exhibited apparent preference to the rewarded (-)-menthol rather than punished vanillin odor after three trials, and induced memory retention for 1-day. Then we simplified the conditioning procedure to analyze the causation of cockroach attractiveness to the rewarded odor. We conducted an elemental conditioning by associating a (-)-menthol or vanillin odor with sucrose reward or saline punishment independently. Increase or decrease in the number of animal visits to the odor source evaluates the results. The number increased after (-)-menthol was presented with reward, while it did not change for rewarded vanillin odor. In both odors, the number decreased significantly after training with saline punishment. These results indicate that cockroaches learn a single odor presented as a conditioned stimulus, although association of odor with reward or punishment depends largely on the chemical. This simplified olfactory conditioning procedure enables further analysis of cockroach searching behavior evoked by a learned general odor.

Keywords:*German cockroach, Olfactory learning, behavior*

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O205TH04

Physiology

O205

Expression profiles and functional study of three odorant binding proteins in *Nilaparvata lugens* Stal

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Odorant binding proteins (OBPs) play important roles in insect olfaction. The brown planthopper (BPH), Nilaparvata lugens Stal (Delphacidae , Auchenorrhyncha, Hemiptera) is one of the most important rice pests. Its monophagy (only feeding on rice), wing form (long and short wing) variation, and annual long distance migration (seeking for rice plants of high nutrition) imply that the olfaction would play a central role in BPH behavior. However, the olfaction related proteins have not been characterized in this insect. In this study, full length cDNAs of three OBPs were obtained by combination use of homologous searching of thranscriptomic data and RACE techniques, and distinct expression profiles were revealed regarding to tissue, developmental stage, wing form and gender for the first time for the species. Further binding assays with 41 compounds demonstrated that NlugOBP3 had markedly higher binding ability and wider binding spectrum than the other two OBPs. Terpenes and Ketones displayed higher binding while Alkanes showed no binding to the three OBPs. Focused on NlugOBP3, RNA interference experiments showed that NlugOBP3 not only involved in nymph olfaction on rice seedlings, but also had non-olfactory functions, as it was closely related to nymph survival. Therefore, it might serve as a potential target for developing behavioral disruptant and/or lethal agent in N. lugens.

Keywords:*The brown planthopper, Olfaction, Wing form, Ligand binding, RNA interference, Rice seeking behavior*

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O205TH05

Physiology

O205

Differential localization of splice variants of a glutamate-gated chloride channel subunit in the housefly

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L-Glutamic acid (glutamate) functions as both excitatory and inhibitory neurotransmitters in the nervous system of invertebrates. The action as the inhibitory neurotransmitter is mediated by glutamate-gated chloride channels (GluCls), which are formed by five subunits encoded by a single gene. It is important to study the physiological roles and the pharmacological properties of GluCls as they are considered important targets for insecticides. In this study we first performed quantitative PCR (qPCR) and immunohistochemical analysis to examine the tissue localization of GluCls in the housefly (Musca domestica L.). The results indicated that GluCls were primarily localized in the optic lobe of the adult head. They were also distributed in the somata of the motor neurons of the thoracic ganglion and nerve fibers in the leg. As alternative splicing of pre-mRNA generates three exon-3 splice variants (A, B, and C) of the GluCl subunit, we next performed qPCR analysis to investigate the expression level of each variant in the adult housefly. The transcripts of variants A and B were primarily expressed in the head, whereas that of variant C was predominantly detected in peripheral tissues such as the leg and the abdomen. When heterologously expressed in Xenopus oocytes, the three variant channels did not show significant differences in sensitivity to glutamate and the maximal amplitude of currents evoked upon activation by glutamate, although the expression levels of three variant channels in Xenopus oocyte were different. These findings suggest that GluCl variants participate in signal transduction in different regions of the housefly.

Keywords:*glutamate-gated chloride channels, splice variant, localization, housefly*

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O205TH06

Physiology

O205

Sequencing and characterizing odorant receptors sensitive to the pheromones of the Cerambycinae (Coleoptera: Cerambycidae)

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Many species of longhorned beetles (Cerambycidae) rely on volatile pheromones to locate mates. Pheromones within subfamilies and genera may be highly conserved, and many species produce similar or identical compounds. In the subfamily Cerambycinae, the chemicals 3-hydroxyhexan-2-one, 2,3-hexanediol, and 2-methyl-1-butanol are produced as pheromones by many dozens of species. We are studying the pheromone receptors of the Cerambycinae to better understand the evolution of this unusual overlap of pheromones. We sequenced an antennal transcriptome of the longhorned beetle Megacyllene caryae (Gahan), which produces in its pheromone blend the common cerambycine pheromones 2,3-hexanediol and 2-methyl-1-butanol, and characterized odorant receptors sensitive to these two pheromones as well as an additional pheromone, 2-phenylethanol. We used degenerate primers to isolate potential orthologs of these receptors from the congener M. robiniae, and we have begun partial genomic sequencing of additional cerambycine species to explore the evolution of pheromone-sensitive odorant receptors within the subfamily.

Keywords:*Cerambycidae, odorant receptor, pheromone, transcriptome*

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O205TH07

Physiology

O205

Lost in translation: Modified olfactory inputs drive male moth behavioral responses in interspecific antennal transplants

Seong-Gyu Lee[1], Kathy R. Poole[2], Charles E. Linn[3], Neil J. Vickers[4]

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Insects rely on their olfactory system to locate food sources, enemies, mates, and oviposition sites. Sex pheromone communication is especially critical for successful reproduction in many insect species. To discriminate the pheromone of potential mates against an environmentally noisy background, precise detection and discrimination of sex pheromone composition by the brain is required. In this study, we modified olfactory inputs to the brain by transplanting antennal imaginal discs between two closely-related species of heliothine moth Heliothis virescens and Heliothis subflexa. Behavioral assays showed that the adult transplant males were still attracted to certain combinations of sex pheromone components but the composition of these blends were not typical of those attractive to normal males of either species. We also examined the physiological and morphological characteristics of peripheral olfactory receptor neurons located on the antenna and second-order olfactory projection neurons in the antennal lobe. The results shed light on how the brain assembles an odor "entity" from discrete odorant-specific inputs. Supported by NSF-IOS 0641014 to NJV and CEL

Keywords:*Sex pheromone, Heiothine moth, Electrophysiology, Transplants*

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O205TH08

Physiology

O205

The overexpression of acetylcholinesterase compensates for the reduced catalytic activity caused by resistance-conferring mutations in *Tetranychus urticae*

Deok Ho Kwon[1], Jae Young Choi[2], Yeon Ho Je[3], Si Hyeock Lee[4]

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The mutations (G228S, A391T and F439W) and duplication of the acetylcholinesterase (AChE) gene (Tuace) are involved in monocrotophos resistance in the two-spotted spider mites, Tetranychus urticae. The overexpression of T. urticae AChE (TuAChE) as a result of Tuace duplication was confirmed in several field-collected populations by Western blotting using an AChE-specific antibody. To investigate the effects of each mutation on the insensitivity and fitness cost of AChE, eight variants of TuAChE were expressed in vitro using the baculovirus expression system. Kinetic analysis revealed that the G228S and F439W mutations confer approximately 26-fold and 99-fold increases in the insensitivity to monocrotophos, respectively, whereas the insensitivity increased over 1165-fold in the AChE with double mutations. To simulate the catalytic activity of the overexpressed TuAChE in two T. urticae strains (approximately 6 copies for AD strain vs. 2 copies for PyriF strain), appropriate TuAChE variants were combined to make up the desired AChE copies and mutation frequencies, and their enzyme kinetics were determined. The reconstituted 6-copy and 2-copy TuAChEs exhibited catalytic efficiency levels comparable to those of a single-copy wildtype TuAChE, suggesting that, if mutations are present, multiple copies of AChE are required to restore a normal level of catalytic activity in the monocrotophos-resistant mites. In summary, the present study provides clear evidence that Tuace duplication resulted in the proportional overexpression of AChE, which was necessary to compensate for the reduced catalytic activity of AChE caused by mutations.

Keywords:*Tetranychus urticae, acetylcholinesterase, gene duplication, mutation, compensation, resistance*

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O206F01

Physiology

O206

A novel mechanism of ecdysone biosynthesis regulation in the prothoracic glands of *Bombyx mori*

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In insect, growth and development are controlled and coordinated by the precisely timed changes of the insect steroid hormone, ecdysone. The ecdysone is predominantly synthesized in the prothoracic glands (PGs) and the biosynthesis is intricately regulated by neuropeptides. However, the whole regulatory mechanisms of ecdysone biosynthesis are not fully understood. To elucidate the entire pathway of ecdysone biosynthesis including its regulatory mechanisms, we performed a transcriptome analysis of the PGs in Bombyx mori using next-generation sequencing (NGS). After the NGS, we further screened the candidates by tissue distribution and expression profile of the genes in B. mori. By these screening, we found a G-protein coupled receptor (GPCR) that is strongly suggested to participate in the regulation of ecdysone biosynthesis. The GPCR gene was predominantly expressed in the ecdysteroidgenic tissues, and the expression pattern in the PGs was in concert with the ecdysteroid titer in the hemolymph. Furthermore, we tested the binding of ligand candidates to the GPCR using a heterologous expression system, and found a ligand. The ligand stimulated the ecdysone biosynthesis in the PGs, but not affected to the gene expression of known ecdysone biosynthesis-related enzymes. In addition, the RNAi phenotype of homologous genes of the GPCR in Drosophila melanogaster showed larval lethal or growth delay. These results suggest the importance of this GPCR mediated novel regulatory mechanism in the ecdysone biosynthesis. [This study was supported by the grant from Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.]

Keywords:*ecdysone biosynthesis, neuropeptide, GPCR*

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O206F02

Physiology O206

A Chitin Deacetylase-Like protein homologue in the spruce budworm, *Choristoneura fumiferana*

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Chitin is a naturally occurring polysaccharide that is one of the main components of insect exoskeletons and peritrophic membrane (PM), foregut and hindgut, as well as tracheal tissue. It plays a critical role in modelling tissues of various shapes, sizes and mechanical properties. Chitin deacetylase-like proteins (CDAs) convert chitin into its deacetylated form, chitosan, a natural glucosamine polymer. CDAs are members of a family of carbohydrate esterases and have been well characterized in pathogenic fungi and bacteria. Recently, insect CDAs have been isolated and characterized from cabbage looper, drosophila and red flour beetle. Insect CDAs were classified into five ortholog groups based on phylogenetic analysis and the presence of additional motifs. Red flour beetle has five ortholog groups. Group I contains two genes. During knock-down of all the nine CDAs individually, only group I CDA knock down exhibited similar lethal phenotypes and developmental blocks in the red flour beetle. A CDA-like gene was isolated during yeast two hybrid screening using the spruce budworm gene CfRACK1 (Receptor of Activated C Kinase 1) as bait. The deduced amino acid sequence, gene structure and expression profile indicated that the cloned gene from spruce budworm is similar to that in group I CDA in red flour beetle. Its biological roles and potential use in pest control are discussed. This research was supported by grants from the Canadian Regulatory System for Biotechnology (CRSB) and the Genomics R&D Initiative (GRDI).

Keywords:*Chitin Deacetylase-Like protein spruce budworm*

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O206F03

Physiology

O206

Structural insight into the funtional and inhibitiory specificity of an insect chitinolytic β-N-acetyl-D-hexosaminidase, a potential pesticide target

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Chitinolytic β-N-acetyl-D-hexosaminidase (Hex) is a branch of GH20 Hex that is only distributed in insects and microorganisms, and is therefore a potential target for the action of pesticides. OfHex1 from the Asian corn borer, Ostrinia furnacalis, functions merely in chitin degradation. In this study, the crystal structures of OfHex1 was determined. The structural basis for the functional specificity of OfHex1 was attributed to the existence of three conserved residues (Val327, Glu328 and Trp490) at the entrance of the active pocket. To guide the design of pesticide against OfHex1, a selective inhibitor (TMG-chitotriomycin) and a broad-spectrum inhibitor (PUGNAc) were co-crystallized with OfHex1 respectively. TMG-chitotriomycin is a natural selective inhibitor against bacterial and insect Hexs. The selective inhibition of OfHex1 by TMG-chitotriomycin was related to the existence of the +1 subsite at the active pocket and a key residue, Trp490, at this site. Mutation of Trp490 to Ala led to a 2,277-fold decrease in sensitivity toward TMG-chitotriomycin. The sensitivities of chitinolytic Hexs towards PUGNAc can vary in 100 folds [OfHex1 (insect)< SmCHB (bacterium)< SpHex (actinomycete)]. The sensitivities of these enzymes to PUGNAc was dedicated by the active pocket size, with OfHex1 having the largest but with the narrowest entrance, while SpHex having the smallest, suitable for holding the inhibitor, whereas that of SmCHB having the widest entrance. By widening the size of the active pocket entrance of OfHex1 through replacing active site Val327 with Gly, sensitivity of OfHex1 to PUGNAc became similar to that of SmCHB.

Keywords:*chitin, hexosaminidase, crystal structure, inhibitor, pesticide*

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O206F04

Physiology

O206

A novel alternative splicing site of class A chitin synthase from the insect *Ostrinia furnacalis*

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Insect chitin synthase A (CHSA) catalyzes chitin biosynthesis in tissues that develop from ectoderm. Since only one gene copy encodes CHSA, we hypothesized that CHSA is very likely to exist as isoforms through alternative splicing, and the functions of these isoforms may be tissue-specific. Besides the known alternative splicing exons in the mid-ORF region, we report here the alternative exons (OfCHSA-2a andOfCHSA-2b) of OfCHSA, the chitin synthase A from the lepidopteran pest Ostrinia furnacalis. Sequence analysis of the 50 upstream region of the transcription start site indicated that presences of two independent promoters for controlling the expression of OfCHSA-2a/b. Both OfCHSA-2a and OfCHSA-2b transcripts were preferentially expressed in the epidermis. During growth and development of O. furnacalis, OfCHSA-2a was mainly expressed during larvalelarval molting and larval-pupal transformation, as well as in newly-laid eggs, while OfCHSA-2b was expressed only during the larval-larval molting. Gene silencing of OfCHSA-2a caused incomplete molting, while silencing of OfCHSA-2b exclusively influenced the head cuticle formation of the 3rd instar larval. Since O. furnacalis is phylogenetically close to the model insect Bombyx mori, the same undiscovered alternative splicing exon was also identified in BmCHSA by gDNA sequence alignment. This work may lead to greater understanding of the mechanism by which a single copy of the CHSA gene could fulfill various functions with tissue specificity.

Keywords:*alternative splicing, chitin synthase, insect, RNAi*

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O206F05

Physiology

O206

Molecular cloning of chitinase cDNAs from the spittlebug *Poophilus costalis* (Hemiptera: Cercopoidea: Aphrophoridae)

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The spittlebug nymphs feed on plant sap and produce forth to cover their fragile bodies. In this study, we have confirmed the fungicidal effect of the froth of the spittlebug Poophilus costalis by bioassay on the pea wilt pathogen, Fusarium oxysporum f. sp. pisi; in addition, the activities of β-N-actylglucosaminidase, chitobiosidase and endochitinase were observed in the froth. Furthermore, a chitinase cDNA, named PCChi, was cloned from the Malpighian tubules of P. costalis nymphs. The PCChi cDNA contains an 1809-nucleotide open reading frame encoding a protein of 603 amino acids with an estimated molecular weight of 68 kDa. The deduced amino acid sequence of PCChi has 58-86% identities to known chitinases in the other species.

Keywords:*Poophilus costalis, spittlebug, chitinase, molecular cloning*

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O206F06

Physiology

O206

Tick ecdysteroidogenesis - Identification of ecdysteroid synthetic tissues from a soft tick

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As in other arthropods, blood feeding ticks require ecdysteroids for development and egg production. During the nymphal stages, blood feeding (engorgement) is a trigger for ecdysteroidogenesis. On the other hand, females require both blood feeding and mating to release ecdysteroids. This indicates that female tick ecdysteroidogenesis is regulated by nutrition and mating. Although studies on tick ecdysteroidogenesis have been carried out in the past, there is little definitive information on ecdysteroidogenesis in ticks, the synthetic organs and enzymes have not been identified. Therefore, the objective of this study was to identify tick ecdysteroidogenic enzymes and synthetic tissues using the soft tick Ornithodoros moubata. Cloning of ecdysteroidogenic enzymes were performed with next generation sequencing and the results revealed two ecdysteroidogenic enzymes: Spook (OmSpo, CYP307A1) and Shade (OmShd, CYP314A1). Spook functions in the ecdysone synthetic tissues and Shade converts ecdysone into active form 20-hydroxyecdysone (20E). OmSpo expression was observed in the ovary and cuticle of fed females. Interestingly, 5th instar nymphs that molt into females also showed OmSpo expression in the disc of the ovary. These results indicate the ovary and gonadal disc are the ecdysteroidogenic tissues of both immature and mature ticks. OmShd expression was strongly observed in the ovary, midgut and malpigulan tubes, but not in the fat body. Therefore, ticks appear to have the similar ecdysteroidogenic pathway as insects and crustaceans, but the synthetic tissues appear to have a unique evolution.

Keywords:*Soft tick, Ecdysteroidogenesis, Reproduction, Halloween genes*

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O206F07

Physiology

O206

Molecular mechanism of pupal coloration in the silkworm, *Bombyx mori*

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Insect body colors exhibit extreme diversity, and vary depending on the species and individuals. The body colors greatly contribute to achieve camouflage or mimicry together with other morphological and behavioral traits, thereby strongly associated with natural selection. The formation of insect body color is controlled by not only genetic diversity but also environmental stimuli. The domesticated silkworm, Bombyx mori, is a good model insect for studying pigmentation system because it has many body color mutants and advantages of easily handling. Two pupal color mutants have been obtained in Bombyx mori. One is black pupa (bp) and the other is sooty (so). Both pupal skin colors are abnormally black and affected by temperature during the wandering stage. The responsible gene of the so mutant is ebony cloned by Futahashi et al. (2008), but the bp mutant is still unknown. Positional cloning of the bp mutation indicated that L-aspartic acid decarboxylase (ortholog of the Drosophila black gene), which catalyzes β-alanine synthesis, was a first candidate for the responsible gene. The pupal skin color of the bp mutant was recovered by direct injection of β-alanine from black to normal (brown). GC-MS analysis also showed depletion of β-alanine in pupal skin of the bp mutant. From these results, we speculate that the L-aspartic acid decarboxylase gene is responsible for the bp mutation. Now, we are trying to clarify the molecular mechanism of pupal pigmentation affected by both genes and external temperature using bp and so mutants as a model.

Keywords:*Bombyx mori, pupal coloration, positional cloning, L-aspartic acid decarboxylase, β-alanine*

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O206F08

Physiology

O206

Eicosanoids mediate hemolymph oxidative and antioxidative response in larvae of *Galleria mellonella*

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Antioxidant enzymes play a major role in the defense against pro-oxidative effects of xenobiotics and prooxidant plant allelochemicals in insects. We posed the hypothesis that eicosanoids also mediate antioxidant enzymatic defense reactions to pro-oxidant challenge. To test this idea, we reared first-instar larvae of Galleria mellonella (L.) with esculetin (0.001%), dexamethasone (0.001%) and phenidone (0.1%) to seventh-instars. Newly ecdysed seventh-instars were then fed on artificial diet containing 0.05% xanthotoxin (XA) for 2 days. High dietary XA concentrations (0.005 and 0.1%) were associated with increasing MDA and carbonyl content (by 3-fold) and antioxidant enzyme activities, superoxide dismutase (SOD) (by 3-fold) and catalase (CAT) (by 4-fold), and glutathione-dependent enzymes, glutathione S-transferase (GST) (by 15-fold) and glutathione peroxidase (GPx) (by 7-fold). Relative to control, eicosanoid biosynthesis inhibitors (EBIs) esculetin, dexamethasone and phenidone also resulted in impaired MDA content and antioxidant enzyme activities. However, carbonyl content did not differ between control- and EBIs-feeding larvae. This oxidative stress was associated with elicited antioxidative responses by increasing SOD, GST and GPx and decreasing CAT activities in hemolymph. We infer from these findings that eicosanoids mediate insect antioxidant enzymatic responses to dietary pro-oxidants.

Keywords:*Eicosanoids, Galleria mellonella, Antioxidant enzymes, Oxidative stress*

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O206F09

Physiology

O206

Post-feeding diuresis in Rhodnius prolixus: The role of FGLamide-related allatostatins

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Allatostatins (ASTs) are insect neuropeptides that were first identified as inhibitors of juvenile hormone biosynthesis by the corpora allata. There are three families of ASTs in insects, defined by their C-terminus conserved regions, one of which is FGLamide. Here we determine, for the first time in a hemipteran, the complete 1013bp cDNA sequence encoding the Rhodnius prolixus FGLa/ASTs (Rhopr-FGLa/ASTs). Reverse-transcriptase PCR demonstrates that the Rhopr-FGLa/AST transcript is highly expressed in the central nervous system (CNS) in unfed fifth-instar R. prolixus, and is reduced in expression in CNS dissected from one day old blood-fed insects. Fluorescent in situ hybridization shows transcript expression in neurons in each ganglion of the CNS, but also in cells located on peripheral nerves. Notably, the transcript is localized in five dorsal unpaired median (DUM) neurons in the mesothoracic ganglionic mass, which produce neurohaemal sites over the abdominal nerves. Pre- and post-feeding analysis of allatostatin-like immunoreactivity shows that the intensity of staining is reduced in DUM neurons, abdominal nerves and the open-type endocrine cells in the anterior midgut at 48 hours post-feeding. Rhopr-FGLa/ASTs dose-dependently inhibit contractions of the anterior midgut and hindgut, and heart-beat frequency. We have also examined their effects on secretion by Malpighian tubules. Rhopr-FGLa/ASTs appear to be released into the haemolymph following feeding and to inhibit the activity of various tissues associated with feeding. Lastly, we have predicted a single receptor for FGLa/ASTs in R. prolixus and phylogenetic analysis classifies it as a type 1 receptor, with two allatostatin receptors present only in Drosophila.

Keywords:*Neuropeptide, Myoinhibition, Visceral muscle, Diuresis*

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O206F10

Physiology

O206

The unexpected divergence of juvenile hormone function in highly eusocial tropical wasps

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New World swarm founding wasps (Epiponini) represent a pinnacle of eusocial evolution, comparable to honeybees and ants. Yet almost nothing is known about their physiology. This is in stark contrast to primitively eusocial wasps like Polistes which have been interrogated for decades with particular emphasis on the role of hormones in fights among foundresses where, typically, one female will dominate and become the sole egg layer. In contrast to Polistes, swarm founding wasps by definition lack foundresses and solitary phases, multiple queen nests are normal, and dominance interactions have become ritualized in the form of abdomen-bending. Here, we report the endocrine profiles of juvenile hormone (JH) and the ecdysteroids for two species of swarm founders with caste-totipotent adults: Polybia micans and Synoeca surinama. In normal or status quo nests, JH levels were virtually identical between queens and workers of P. micans. In contrast, S. surinama queens showed significantly higher JH titers than workers. Changes in JH titers in both species were nonetheless associated with the transition from young workers to queens in queenless nests. Additional assays performed for S. surinama in a variety of social contexts revealed that JH titers rise following abdomen-bending. Topical application of either methoprene (a JH mimic) or JH III under various circumstances confirmed that JH does not drive the transition but may have a gonadotropic and queen-signaling function. These results demonstrate that the underlying endocrinology of eusocial wasps is much more diverse and labile than expected. Supported by HHMI, CNPq and FAPESP 10/10027-5

Keywords:*juvenile hormone, wasps, eusocial*

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O206F11

Physiology

O206

Female-specific wing regression triggered by 20-hydroxyecdysone during pupal-adult transformation in the winter geometrid moth *Nyssiodes lefuarius* (Lepidoptera, Geometridae).

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The winter moth Nyssiodes lefuarius is a univoltine species, and the adults appear at early spring after long pupal "diapause" from summer to winter. They show sexual dimorphism in wing form: Males have functional wings of normal size, whereas females lack wings. I previously found that the wing discs of female pupae in N. lefuarius disappeared due to apoptosis during pupal-adult transformation, while those of males continued to develop fully into adult wings. Using light and electron microscopy, further, it was revealed that wing regression of females is involved not only in apoptotic cell death and phagocytosis, but also accompanied by epithelial cell contraction. In the present investigation, to know whether ecdysteroid is involved in such sex-specific wing regression, I tested in vivo condition the effects of 20-hydroxyecdysone (20E) on pupal diapause termination and wing morphogenesis in both sexes. When 20E of 5.4 μg or more was applied, wing morphogenesis began after 4 days of injection and then wing regression was found in females. In males, by contrast, wing morphogenesis including scale formation was induced. These results suggest that ecdysteroid plays important roles in sex-specific wing regression in this geometrid moth.

Keywords:*winter moth, apoptotic cell death, wing regression, 20-hydroxyecdysone, sexual dimorphism*

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O206F12

Physiology

O206

Regulatory mechanism of the *Drosophila* insulin-like peptides gene expression

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Members of the insulin-like peptides have conserved roles in the regulation of growth and metabolism in a wide variety of metazoans. The Drosophila genome encodes seven insulin-like peptide genes, dilp1?7, and the most prominent dilps (dilp2, dilp3 and dilp5) are expressed in brain neurosecretory cells known as insulin-producing cells (IPCs). Although these dilps are expressed in the same cells, the expression of each dilp is regulated independently. Furthermore, the expression of one of the three genes, dilp5 is tightly regulated by nutrient availability. However, the molecular mechanisms that regulate the expression of individual dilps in the IPCs remain largely unknown. Here, we show that Dachshund (Dac), which is a highly conserved nuclear protein, is a critical transcription factor that specifically regulates dilp5 expression. Dac was strongly expressed in IPCs throughout development. dac loss-of-function analyses revealed a severely reduced dilp5 expression level. Dac interacted physically with the Drosophila Pax6 homolog Eyeless (Ey), and these proteins synergistically promoted dilp5 expression. In addition, the mammalian homolog of Dac, Dach1/2, facilitated the promoting action of Pax6 on the expression of insulin in cultured mammalian cells. These observations indicate the conserved role of Dac/Dach in controlling insulin gene expression in conjunction with Ey/Pax6 (Okamoto et al., PNAS 2012). Although we revealed the molecular mechanism of dilp5 expression by two transcription factors Dac and Ey, we still do not know how these proteins involve in the nutrient-dependent regulation. In this presentation, we will also show that the molecular mechanism underlying the nutrient-dependent expression of dilp5.

Keywords:*Insulin-like peptides, Drosophila, neurosecretory cells, Dachshund, Eyeless, Nutrition*

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O206F13

Physiology

O206

Molecular characterization and gene expression of juvenile hormone binding protein in the Bamboo Borer, *Omphisa fuscidentalis*

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In this study, we cloned and sequenced the Omphisa juvenile hormone binding protein (OfJHBP). The full-length OfJHBP cDNA sequence is comprised of 849 nucleotides with an open reading frame of 726 bp encoding 242 amino acids. The molecular mass of the protein was estimated to be 26.94 kDa. The deduced protein sequence of OfJHBP showed moderate homology with the lepidopteran, Heliothis virescens JHBP (52% amino acid identity) and lower homology with the Bombyx mori JHBP (45%) and the Manduca sexta JHBP (44%). The OfJHBP was expressed mainly in the fat body. OfJHBP transcripts in the fat body was moderately high during 3rd, 4th and 5th instars, then rapidly increased, reaching a peak during early diapause. The expression remained high in mid-diapause, then decreased in late-diapause until the pupal stage. Application of juvenile hormone analog (JHA) methoprene to diapausing larvae stimulated OfJHBP mRNA expression in the fat body from day 2 after hormone application and reached maximum levels on day 10, then declined to low levels in the pupal stage. The changes of OfJHBP mRNA expression level after 20-hydroxyecdysone (20E) injection revealed that 20E also activated OfJHBP mRNA expression level. OfJHBP mRNA levels increased on day 2 and showed a peak of gene expression on day 6, and decreased to low levels during pupation. Thus, both hormones exhibited a similar stimulatory pattern in OfJHBP expression.These results should contribute to our understanding of how JHBP and JH regulate the termination of larval diapause in the bamboo borer.

Keywords:*bamboo borer, juvenile hormone binding protein, cDNA cloning, juvenile hormone, 20-hydroxyecdysone*

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O206F14

Physiology

O206

Understanding physiological roles of crustacean cardioactive peptide in the blood-gorging bug, *Rhodnius prolixus*

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Crustacean cardioactive peptide (CCAP), a cyclic nonapeptide (PFCNAFTGCamide), has multifunctional roles in insects including the stimulation of visceral and cardiac muscle contraction, and the regulation of ecdysis. We have cloned the cDNA sequence of the CCAP gene from Rhodnius prolixus central nervous system (CNS), and shown its expression in the CNS using in situ hybridization. The amino acid sequence of CCAP in R. prolixus CNS was verified by matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS) and MALDI-TOF MS/MS. The distribution of CCAP containing neurons and their processes was also shown using immunohistochemistry. In addition, we have sequenced the cDNA of the RhoprCCAP G-protein coupled receptor (GPCR) and examined its expression in the CNS as well as peripheral tissues of R. prolixus. Quantitative real-time reverse transcriptase PCR (qRT-PCR) analysis showed specific developmental expression of CCAP and its receptor in the CNS and peripheral tissues of 4th and 5th instar R. prolixus. Physiologically, CCAP dose-dependently stimulates R. prolixus hindgut contractions, with threshold at 5x10-9 M and maximum response at10-7M CCAP. Also, CCAP increases heartbeat frequency in a reversible, dose-dependent manner, with threshold close to 10-11M and maximum response at 10-10M CCAP. The functional roles of CCAP in ecdysis in vivo are currently being investigated by injecting double stranded RNA (dsRNA) generated by using CCAP and its receptor as templates.

Keywords:*Insect , CCAP, Physiology, Immunohistochemistry, qPCR, RNAi*

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O206F15

Physiology

O206

Transcriptional mechanism of Kruppel homolog 1, a repressor of insect metamorphosis, in *Bombyx mori*

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Abstract Title: Kruppel homolog 1 (Kr-h1) plays a key role in the repression of metamorphosis in insects. Kr-h1 is assumed to be induced by Juvenile hormone (JH) via a JH receptor, Methoprene-tolerant (Met), but the mechanism of induction is largely unknown. To gain insights into the molecular mechanism of Kr-h1 induction, we first cloned cDNAs encoding Kr-h1 (BmKr-h1) and Met homologs (BmMet1 and BmMet2) from Bombyx mori. In a B. mori cell line, BmKr-h1 was rapidly induced by subnanomolar levels of natural JHs. A novel JH response element (kJHRE), comprising 141 nucleotides, was discovered at 2 kb upstream from the transcription start site of BmKr-h1 by reporter assays in the B. mori cells. The core region of kJHRE (kJHREc; GGCCTCCACGTG) contained a canonical E-box sequence, to which the Met, a bHLH transcription factor, is likely to bind. In mammalian HEK293 cells, which lack an intrinsic JH receptor, ectopic expression of BmMet2 fused with Gal4DBD induced JH-dependent activity of a UAS-reporter. Meanwhile, the kJHRE-reporter was activated JH-dependently in HEK293 cells when co-transfected with BmMet2 and BmSRC, a steroid receptor coactivater, suggesting that BmMet2 and BmSRC jointly interact with kJHRE. We also showed that BmMet2 JH-dependently interacted with BmSRC. Based on these results, we suggest the mechanism of JH-mediated induction of BmKr-h1 as follows: BmMet2 accepts JH as a ligand, JH-liganded BmMet2 interacts with BmSRC, and JH/BmMet2/BmSRC complex activates BmKr-h1 gene via an interaction with kJHRE.

Keywords:*juvenile hormone, JH response element, methoprene tolerant, steroid receptor coactivator, insect growth regulator*

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O206F16

Physiology

O206

Insulin signaling is involved in nutritional stress response in pea aphids, *Acyrthosiphum pisim*

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Researchers have identified the insulin/IGF-like (IIS) signaling pathway as the major signaling pathway coordinating growth and regulating nutritional plasticity in diverse organisms. Less well understood at the molecular level is how organisms adjust their nutritional metabolism in response to the environmental stress. The publication of draft sequence of the pea aphid, Acyrthosiphum pisim, laid a foundation for revealing the relationship between IIS pathway and nutritional assignment under nutrition-related adversity; in particular for winged morphs that are closely with migration. Like hymenopterans, two insulin receptor genes are present in the pea aphid genome, but there are no any experimental data about them in aphids. To elucidate the role of the IIS pathway in pea aphid, two putative insulin receptor genes (AInR-1 and AInR-2) were choose for analysis of gene expression profiles under nutritional deficiency stress. We show that IIS pathway is associated nutritional stress response. Not only winged and wingless adults differ in the level of insulin receptor genes expression when coping with negative nutritional condition, but the scale of expressional changes of two receptors genes is different. These data indicate that IIS pathway presents different modulatory patterns according to tasks that wing dimorphism gives diverse morphs under adverse nutritional condition. And in the progress, the relationships between both insulin receptors may be coordination, but probably one plays a leading role among them.

Keywords:*IIS pathway, insulin receptor, gene expression, nutrition, dimorphism*

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O206F17

Physiology

O206

Queen pheromone regulates egg production in a termite

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In social insects, resource allocation is a key factor that influences colony survival and growth. Optimal allocation to queens and brood is essential for maximum colony productivity, requiring colony members to have information on the total reproductive power in colonies. However, the mechanisms regulating egg production relative to the current labour force for brood care remain poorly known. Recently, a volatile chemical was identified as a termite queen pheromone that inhibits the differentiation of new neotenic reproductives (secondary reproductives developed from nymphs or workers) in Reticulitermes speratus. The same volatile chemical is also emitted by eggs. This queen pheromone would therefore be expected to act as an honest message of the reproductive power of queens. In this study, we examined how the queen pheromone influences the reproductive rate of queens in R. speratus. We compared the number of eggs produced by each queen between groups with and without exposure to artificial queen pheromone. Exposure to the pheromone resulted in a significant decrease in egg production in both single-queen and multiple-queen groups. This is the first report supporting the role of queen pheromones as a signal regulating colony-level egg production using synthetically-derived compounds in a termite.

Keywords:*queen pheromone, egg production, termites, resource allocation*

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O206F18

Physiology

O206

Analysis of an Innate behavioral sequence by genetic manipulation of a peptidergic signaling cascade

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Insect ecdysis is an example of innate behavioral sequences performed as a fixed action pattern. The release of ecdysis-triggering-hormone (ETH) from endocrine Inka cells drives the stereotypical sequence of ecdysis-related behaviors through direct and sequential activation of peptidergic ETH receptor ensembles in the central nervous system (CNS). Here we show that targeted inactivation and ablation of ETH receptor (ETHR) neurons, including those that express kinin, crustacean cardioactive peptide (CCAP), Allatostatin-CC (Ast-CC), myoinhibitory peptide (MIP), burs, and bursicon (burs and pburs) alter scheduling of the ecdysis sequence. The results demonstrate that kinin is involved in scheduling of pre-ecdysis and that bursicon neurons are necessary for termination of pre-ecdysis and the onset of ecdysis. Concurrent monitoring of neuronal activity by calcium imaging in kinin and bursicon neurons provides compelling evidence for sequential activation of ETH receptor neurons and functional roles of these neurons in ecdysis. Evidence supporting this model is provided through over-expression or silencing of ETHR in kinin and bursicon neurons, and by observations of changes in behavioral scheduling that result from these manipulations. Changes in onset timing of bursicon neurons by over-expression or silencing ETHR were confirmed by calcium imaging. To explain the sequential activation of ETHR neurons, a differential sensitivity model is proposed: variable sensitivity to ETH causes differences in activation timing of ETHR ensembles. Our findings provide new evidence for functional roles of peptidergic ETHR neurons and mechanisms of sequential activation of peptidergic ensembles to schedule steps of the stereotypic ecdysis behavioral sequence.

Keywords:*Innate behavior, Ecdysis triggering hormone*

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O206F19

Physiology

O206

Osmoregulation in the desert locust *Schistocerca gregaria*, the role of ion transport peptide.

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Ionic and osmotic regulation in most insects is ultimately achieved through the selective and controlled reabsorption of solutes and water in the hindgut (ileum and rectum) from fluid secreted by Malpighian tubules. In locusts, the control of Malpighian tubule fluid secretion by diuretic hormones is well established, far less is known of the regulation of reabsorptive processes in the hindgut. Ion transport peptide (ITP), a member of the crustacean hyperglycaemic hormone family, stimulates the uptake of ions (chloride, potassium and sodium), with a concomitant movement of fluid, across the locust ileum in vitro. These processes are mimicked by endogenous cyclic AMP, suggesting that signal transduction is via this second messenger. Acid secretion in the ileum is inhibited by ITP, but not cyclic AMP, implying the involvement of a second receptor and signal transduction pathway. The latter may be via cyclic GMP because this cyclic nucleotide also stimulates ion transport across the ileum, though its effects on acid secretion have yet to be investigated. The involvement of these second messengers in the ITP response will be discussed. Ion and fluid reabsorption across the locust rectum are also stimulated by cyclic AMP and chloride transport stimulating hormone, a peptide partially purified from locust corpora cardiaca. In contrast, ITP has little effect on the rectum, and its physical properties differ to those of CTSH, suggesting reabsorptive processes in the ileum and rectum are differentially regulated.

Keywords:*antidiuresis, signal transduction, ion transport*

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O206F20

Physiology

O206

Orcokinins are conserved peptides in innervation of the salivary gland and rectal sac of ixodid ticks

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Orcokinins (ORC) belong to a family of neuropeptides conserved within Arthropoda, but their cellular distribution and functions in insects and arachnids are inadequately studied. We employed an antibody against conserved N-reminus of ORC (NFDEIDR) and/or in situ hybridization to analyze cells producing ORC-like substances in various insects (Orthoptera, Hymenoptera, Lepidoptera, Diptera) and in the hard ticks (Acarina: Ixodidae). ORC are distributed in specific neurosecretory cells and interneurons throughout the central nervous system in all studied arthropods. In the ticks (Amblyomma variegatum, Dermacentor reticulatus, Hyalomma anatolicum, Ixodes scapularis, I.ricinus, Rhipicephalus appendiculatus) antibody against ORC labeled two pairs of prominent opisthosomal neurons (SG neurons) that innervate both, the salivary glands and the hindgut. ORC immunoreactive axon terminals exclusively reach only type II salivary aciny and form dense network on the surface of the rectal sac. Intensity of immunoreactivity in the SG neurons axon terminals decreased in the course of feeding in female ticks. Mature forms of orcokinins were detected in synganglia from an unfed I. scapularis female by matrix-assisted laser desorption/ionization-time of flight (MALDI TOF/TOF). Our data indicate that orcokinins may control activity of salivary glands and rectal sac from the synganglion. This study was supported by the Research & Development Operational Program funded by the ERDF (ITMS: 26240220044).

Keywords:*neuropeptide, Ixodidae, salivary gland, orcokinin*

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O206F21

Physiology

O206

Comparative proteomical analyses of *Anticarsia gemmatalis* and *Spodoptera frugiperda* midguts

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Anticarsia gemmatalis and Spodoptera frugiperda (Lepidoptera: Noctuidae) are serious defoliator of soybean in all word. In order to better understand the digestion process of such pests, this report shows the evaluation of midgut proteins from A. gemmatalis and S. frugiperda by proteomic analyses combining 2D gels and mass spectrometry. Midguts were removed from larvae and total proteins (approximately 650 µg) were extracted and further submitted to 2-DE. Two-dimensional gels from S. frugiperda and Anticarsia gemmatalis analyzed 206 and 385 spots, respectively. Proteins were identified by MS/MS including catalase, enolase, glyceraldehyde-3-phosphate dehydrogenase, arginine kinase, pyrophosphatase and farnesoic acid O-methyl transferase. All proteins identified seem to affect insect development in different ways, being directly involved in their growth or used as an intermediate in metabolic pathways thatmay influence pest development. According our knowledge, this is the first work using two-dimensional electrophoresis associated to mass spectrometry analyses that evaluates the midgut protein expression of both insect-pests. The identification of those proteins could contribute to the understanding of pest physiology leading to the development of novel strategies for pest management. Key words: Anticarsia gemmatalis, Spodoptera frugiperda, Proteomics, Mass spectrometery.

Keywords:*Anticarsia gemmatalis, Spodoptera frugiperda, Proteomics, Mass spectrometery.*

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O206F22

Physiology

O206

JH acid methyl transferase genomics and its relevance as insect biopesticidal agent

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Corpus allatum (CA) the insect endocrine gland that synthesize juvenile hormone (JH), the sesquiterpenoid is the insect morphogenetic hormone that determines the kind of moult that any of the developing insect has to undergo during its developmental process. The enzyme JH acid methyl transferase (JHAMT) being the rate limiting enzyme in the JH biosynthesis process which in turn obviously determines the circulating JH hormone titer at any specific developmental stage hence obviously becomes a critical factor in the processes of development and morphogenesis. Hence targeting JHAMT enzyme in devising endocrine based biopesticides becomes logical and rewarding. Insect JHAMT gene has been cloned and characterized only in a few insect species. We have made a partially successful attempt on these lines mainly focusing to characterize, clone and sequence the insect JHAMT gene family from two different insect species as the silkworm B. mori (Bivoltine CSR2 X CSR4 hybrid strain) and the Mormon butterfly, Papilio polytes in the absence of its available detailed information. Partial sequence (600bp) of P. polytes JHAMT gene on BLAST analysis showed more affinity in sequence similarity towards that of B. mori even contrary to other known sequence data of related papilio species and even multiple sequence alignment using ClustalW showed that the partial JHAMT sequence from P. polytes is exactly similar to that of B. mori JHAMT gene sequence published in the NCBI data base by Shinoda (2003) even though no difference noticed at 762 and 864 position as found in B.mori bivoltine CSR2 X CSR4. ExPASy

Keywords:*JHAMT Gene, Papilio Polytes, JH acid, Lepidoptera*

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O301TU01

Ecology

O301

Baseline study on diversity, ecology and spatial characterization of invertebrates in and around Lake Tana Sub-basin, Northwestern Ethiopia

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A baseline study was conducted in and around Lake Tana sub-basin, Northwestern Ethiopia to assess the current status of invertebrates involving the relevant stakeholders in biodiversity conservation. The study focused on the role of invertebrates as components in trophic levels, as indicators of climate change, in minimizing pollution and contribution in sustainable ecosystems management. The baseline study covered 4 aquatic, 4 riparian, 3 wetlands and 4 terrestrial sampling sites, respectively in and around the sub-basin. Accordingly, 16 insect and 6 non-insect orders and 103 insect families and 11 non-insect families were collected, identified and preserved, respectively. The total specimens collected and identified by sampling site included, 585 Aquatic and riparian, 1193 wetlands and 550 terrestrial ecosystems making the over all total of specimens collected, identified and preserved 2328, respectively. The results of the study have shown that the degree of organic pollution due to improper and unlimited release of effluents from nearby Hospitals and hotels combined with that from the municipality poor drainage systems are of concerns. The presentation will focus on attributes of invertebrates as natural resources and on ways invertebrates could be best used as indicators of conditions in various ecosystems. The presentation will flash light on the significance of invertebrates in enhancing biodiversity visa-a-vis food security and livelihoods against the back up of climate change and variability

Keywords:*invetebrates,ecosystems*

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O301TU02

Ecology

O301

Biodiversity of stream insects in the Malaysian Peninsula: Spatial patterns and environmental constraints

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The spatial variation in Ephemeroptera, Plecoptera and Trichoptera (EPT) taxonomic composition in headwater forested streams of peninsular Malaysia was studied. The environmental parameters were assessed for their importance to regulate the assemblage composition across the spatial extent of peninsular Malaysia bioregion. The spatial variables were expressed as vectors based on principal coordinates of neighbor matrices (PCNM). The forward selection was based on two stopping criteria to identify the key environmental and spatial variables controlling the EPT taxonomic composition. Eigenvector-based spatial filtering was employed to investigate spatial patterns in the EPT composition, and the variation partitioning technique was applied to estimate the amount of variation in the EPT taxonomic composition attributable solely to environmental and spatial factors. The forward selection retained five parameters (altitude, BOD, velocity, ammonia and DO). These environmental variables explained 13.2% of the total variance in EPT composition. The three spatial variables (PCNM vectors) obtained after forward selection explained 8.4% of composition variation. However, the spatially-structured environmental variables explained 7.5 % of the total variance in assemblage composition of EPT. Altitude was correlated to broad-scale variation and showed to be the most important factor affecting the EPT composition in peninsular Malaysia. Our study revealed spatially-structured patterns of the EPT composition, showing decrease from southern to northern of peninsula Malaysia. The importance of environmental variables in explaining the EPT richness at large spatial scale was highlighted. Our results emphasize the need for spatially-explicit, regional studies to better understand geographical variation of tropical freshwater biodiversity.

Keywords:*EPT, spatial variation, PCNM, peninsular Malaysia.*

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O301TU03

Ecology

O301

Ecological Interactions between non-native and native lady beetles in Maine, the United States

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Biological invasions are currently one of the primary conservation concerns, rivaled only by habitat destruction by humans. In particular, establishment of exotic lady beetles (Coleoptera: Coccinellidae) may have adverse affects on native lady beetle species. We analyzed changes in coccenillid community inhabiting potato crops (Solanum tuberosum L.) in northern Maine over the past 31 years using records from unsprayed plots maintained at the university research farm. Populations of the two native species, Coccinella transversoguttata Brown and Hippodamia tredecimpunctata (Say) seriously declined following the establishment of exotic species Coccinella septempunctata L., Harmonia axyridis (Pallas), and Propylea quatordecimpunctata (L.). A follow-up survey conducted in different habitats in Maine confirmed that C. transversoguttata and H. tredecimpunctata made up only 0.07% and 1.09% of the total lady beetles collected, respectively. Native species were found in very low numbers in all of the habitats surveyed, and in a higher proportion in agricultural habitats when compared to non-agricultural habitats. In laboratory arenas, H. axyridis was the most successful aphid predator, being able to find aphids more quickly and consume more of them compared to most other lady beetle species. It was also by far the most aggressive of the tested species. H. axyridis was also the most successful forager in the presence of the highly aggressive aphid-tending ants Myrmica rubra L. Furthermore, H. axyridis seemed to be highly tolerant of ant venom.

Keywords:*biological invasions, insect conservation, Harmonia axyridis*

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O301TU04

Ecology

O301

Winter-flooded rice cultivation promotes biodiversity of aquatic insects. - Comparisons under different cultivation management systems in Shiga, Japan

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We surveyed aquatic insect communities in paddy fields to examine effects of differences in the cultivation management in Shiga Prefecture, Japan. We set up following four types of paddy fields under different cultivation management systems: conventional management with an application of standard amount of agrochemicals and being kept dry during winter (CV), reduced-agrochemical management and being kept dry during winter (RD), no agrochemical management and being kept dry during winter (OG), and no agrochemical management and being flooded during winter (WF). We selected three orders of aquatic insects: Odonata, Hemiptera, and Coleoptera as targets. In WF paddies aquatic insects were most abundant both in the number of species and individuals throughout the census period. This suggests that winter-flooded managements promoted biodiversity of aquatic insects. In contrast, some species such as Sympetrum was less abundant in WF paddies, indicating their low suitability as the habitats for the nymphs. This is assumed to be due to strong predation pressure, because there were abundant aquatic insects as carnivores in WF paddies. Both in CV and RD paddy fields, aquatic insects were fewer in terms of both the number of species and individuals. This was largely due to the enhanced mortality of the aquatic insects, which was assumed to be killed by agrochemicals used in the early season.

Keywords:*agrochemicals, dragonfly, paddy field*

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O301TU05

Ecology

O301

Parasitoid and pollinator networks in an Australian agro-ecosystem

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Insect pollinators and aphid-parasitoid wasps provide two very important ecosystem services in agro-ecosystems. We collected insect pollinators and aphid parasitoids in farmed and non-farmed habitats, with the aim of assessing if these services cross-over between habitats. Pollinators were collected via vane traps and hand collections, and pollen was swabbed from their bodies to assess their movements between habitats. Aphids and parasitoids invade crops each growing season, presumably from surrounding vegetation. Preliminary results show that aphid and parasitoid populations fluctuated a great deal within the farmed habitats, between years, whilst their populations were fairly similar between years within the non-farmed habitats. Aphidius ervi was found in large numbers in pea aphids on medicago denticulata in the non-cropped habitat, and it has the potential to control the rose-grain aphid which is the common pest aphid of barley crops. However, we did not find evidence of cross-over between farmed and non-farmed habitats, although there is the potential for this to occur. Apis melifera was the most common insect pollinator in both farmed and non-farmed habitats. It did not appear to discriminate between native and non-native plants. 19 Native bee species were recorded, and one new species was discovered. Pollen swabbing revealed cross-over between farmed and non-farmed habitats.

Keywords:*networks*

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O301TU06

Ecology

O301

Cryptic species and putative Mullerian mimicry conceal the role of biogeography in the diversification of Danaus butterflies

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By most accounts, the pantropical butterfly genus Danaus has 5 Australasian, 1 Australasian/African, and 6 New World species, including the migratory Monarch butterfly, D. plexippus. To identify the sister species of the Monarch, we reconstructed the evolutionary history of the genus using ~3,300 bp from four molecular markers sequenced from each of >70 specimens from 11 conventionally accepted species (D. plexaure was unavailable). Results from parsimony, maximum likelihood, and Bayesian methods were strongly supported and largely concordant, revealing that 1) the sister species of the Monarch is not D. erippus as often suggested; 2) D. petilia is genetically distinct from its sister, D. chrysippus; and 3) three Australasian species are polyphyletic as currently circumscribed, requiring taxonomic changes. The geographical distributions of these newly recognized species in the Indo-Australian Archipelago suggest an important role for Wallace's Line and Lydekker's Line as dispersal barriers for these volant insects. Moreover, visual comparisons reveal striking similarity in wing color, pattern, and shape among co-distributed Danaus, suggesting that some species or subspecies may be Mullerian mimics of each other.

Keywords:*biogeography, evolution, Lepidoptera, phylogenetic systematics, Southeast Asia*

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O301TU07

Ecology

O301

Revisiting the ants of Melanesia and the taxon cycle: Historical and human-mediated invasion of a tropical archipelago

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In synthesizing his work on the Melanesian ant fauna, E. O. Wilson proposed a hypothesis for biodiversity dynamics in space and time- the taxon cycle. For the first time since Wilson's landmark studies, we test predictions of a Melanesian taxon cycle with a new dataset on habitat distributions of the entire Fijian ant fauna (183 spp.), and a phylogenetic analysis of the hyperdiverse genus Pheidole in the Indo-Pacific. We find that after colonization, lineages shift from marginal to interior habitats, from ecological generalism to specialization, and from high to low population density, consistent with a historical taxon cycle. Interestingly, exotics are following a similar pathway to these older natural invasions and now dominate the marginal habitats, which may be the first phase of an incipient global taxon cycle in ants.

Keywords:*biogeography, ecology, macroevolution, ants, pheidole*

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O301TU08

Ecology

O301

Managing riparian margins for biodiversity benefits

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Current restoration of stream and other lotic freshwater systems to 'good ecological status' has focused on the creation of riparian buffer strips. Although primarily established to reduce nutrient and sediment run-off from agricultural land, buffer strips are perceived as having multiple benefits including for biodiversity. This study aimed to assess the effects of buffer strips on biodiversity within three river catchments in north-east Scotland. Carabid beetles and pollinators (bees, butterflies and hoverflies) were chosen as biodiversity indicators. Field observations on forty-one buffer strips revealed that, in the short to medium term, buffering creates a homogenous sward atypical of good quality riparian habitat with few truly riparian species present. Studies on carabid beetle movement demonstrated a correlation between activity and vegetation structure. Finally, an experimental approach suggested that cropping or low-intensity grazing offer more sustainable methods of managing these margins. Failure to improve these riparian habitats by buffering alone is because intensive agricultural landscapes do not have sufficient diversity of habitats nor diversity of structure within habitats, to allow colonisation and persistence of a wider suite of species to be promoted. Furthermore, buffering has not addressed the broken linkages between the riparian hinterland, the river banks and the natural flow regime. Restoration of riparian margins is likely to require a range of measures at a catchment scale and over a longer timeframe.

Keywords:*Riparian, Carabidae*

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O301TU09
Ecology

O301

Roles of Insects in the Mesozoic Ecosystems of Northeastern China

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Insects have been in existence for at least 400 million years. These relatively small-sized terrestrial arthropods, equipped with their durable cuticle, allowed them to be preserved in compression fossils and ambers. Based on insect fossils collected in northeastern China (i.e. Liaoning, Inner Mongolia and Hebei), at least 19 orders and more than 300 species have been reported (Ren et al. 2010, 2012). Fossil findings and associated studies in China have made significant progress and contribution to Paleoentomology in recent years. Many insects have close interactions with plants, as demonstrated in many Mid-Mesozoic cases. Protonemestrius jurassicus, a dipteran, had a long tubular mouthpart, suggesting its feeding of sugary fluids, which is important for the study of the origin of angiosperms (Ren, 1998). A probable pollination mode before angiosperms was proposed based on three clades of Eurasian, long-proboscid fossil mecopterans from the Middle Jurassic to the Early Cretaceous, which fed on ovular secretions of extinct gymnosperms and engaged in pollination mutualisms with gymnosperms (Ren et al. 2009). Two extraordinary fossil lacewings (Neuroptera) demonstrate a pre-angiosperm origin for leaf mimesis, revealing a lost evolutionary scenario of interactions between insects and gymnosperms (Wang et al., 2010). Recently, a Jurassic katydid (Orthoptera) was described, which had the stridulatory files on wings to produce low-pitched musical songs to attract females in the mid-Jurassic forest (Gu et al. 2012). Based on the coexisting plants and other animals, we established a structure of the ecosystems for the Yanliao Biota and Jehol Biota respectively.

Keywords:*Insects, fossils, ecosystems, China*

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O301TU10

Ecology

O301

Influence of the river regulation works on ground beetle assemblages in riverbeds of the Yodo River flowing through northern Osaka Prefecture, central Japan

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Landscapes of the Yodo River have been heavily changed since 1971 by river regulation works such as dam construction and extensive excavating. A field survey of ground beetle assemblages (Carabidae and Brachinidae) using unbaited pitfall trapping was carried out in the riverbed of the Yodo River from April to December, 2009 to elucidate the influence of the works on the assemblages. Ground beetles were captured for a week twice a month at 13 sites, where we measured four environmental parameters, riverbed conditions (minor or major bed), submergence period, soil moisture percentage and median particle diameter, at each sites. As a result, a total of 8,697 individuals of ground beetles belonging to 65 species were captured from the whole sites. According to the DCA analysis, 14 species including Harpalus crates, Oodes vicarius and Pterostichus eschscholtzii were ordered at scores of more than 1.0 on axis 1 with 13 species like Synuchus nitidus, Carabus yaconinus and Lesticus magnus lower than -1.0. Moreover, the results of the GLMM revealed that many of former species were related to minor bed and high frequency of submergence duration, and many of latter species were related to major bed and ground aridification. Thus the ground beetles in the riverbed of the Yodo River could be classified into two groups from the result of DCA, showing influence of aridification and/or stability of the riverbed due to the river regulation works.

Keywords:*Carabid beetle, river regulation, pitfall trap, DCA, GLMM*

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O301TU11

Ecology

O301

Effects of plantation management on scarabs feeding on eucalypt and understory grass host plants.

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Management practices can influence insect populations through numerous mechanisms including changes in host plant chemistry and the community dynamics of other plants in the system. Eucalypt plantations account for 90% of the 100 million ha of forest and woodland in Australia and are becoming of considerable commercial importance globally. In a large-scale field experiment, involving 2560 E. saligna trees, we investigated the individual and combined effects of irrigation and fertilisation on scarabs associated with E. saligna. These scarabs typically feed on eucalypt leaves as adults and on the roots of grasses in the understory and adjacent grassland habitats as soil-dwelling larvae. Scarab populations were dominated by Sericesthis nigrolineata (67.5%), Christmas beetles (Anoplognathus porosus) (18.1%) and the Argentine scarab (Cyclocephala signaticollis) (11.31%). We found that irrigation increased adult scarab abundance by 52.3%, increased understory grass cover from 0.47m m-2 to 0.63m m-2 and influenced which grass species were present. There was generally a negative correlation between grass cover and density of larvae in the soil, however some patches with high grass cover contained large numbers of larvae, potentially suggesting preferential oviposition. We suggest that scarab populations were influenced by the effects of irrigation, which determined host plant quality and the nature of plant assemblages generally (e.g. canopy cover, species composition). In complimentary laboratory experiments, we determined whether the dominant scarab, Sericesthis nigrolineata, would change host preferences on the basis of host plant nutritional quality (e.g. primary and secondary compounds) and found that adults readily shift from E. saligna to E. grandis.

Keywords:*Scarab, Feeding behaviour, Population ecology*

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O301TU12

Ecology

O301

Diversity, distribution and plant association of thrips belonging to *Frankliniella* genus in East Africa

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The genus Frankliniella Karny is the second largest in the family Thripidae with many polyphagous thrips and tospovirus vectors which are Neotropic and Nearctic in origin. The invasive Western Flower Thrips (WFT), Frankliniella occidentalis (Pergande) and F. schultzei Trybom are known to occur in East Africa. However detailed information on the distribution and diversity of Frankliniella sp. is lacking in East Africa. Detailed surveys in over 250 locations were undertaken from August 2008 to July 2010. Both the dark and pale forms of WFT were found restricted to the high and mid altitude regions of Kenya. Frankliniella schultzei, the most widespread species was observed in high-, mid- and low altitude regions of East Africa. Both pale forms with short third ocellar setae (OSIII) and dark forms with long OSIII were observed in East Africa. Molecular analysis of the ITS1/ITS2 sequences of F. schultzei forms with RFLP technique indicated high degree of divergence between them. While WFT and F. schultzei were frequently pest on French beans, tomato and capsicum; the latter was also pest on potato, egg plant, cucurbits, amaranthus, pigeon pea etc. For the first time we observed the occurrence of Frankliniella williamsi Hood 1915 and Frankliniella borinquen Hood, 1942 from East Africa. While F. williamsi was frequently observed on graminaceous plants such as maize, sorghum, rice and wheat, F. borinquen was frequently observed on the weed, Tithonia diversifolia (Hemsl.) A Gray. Further detailed information on the plant-association, distribution will be presented in this paper.

Keywords:*Frankliniella, East Africa, Frankliniella occidentalis, Frankliniella schultzei, Frankliniella williamsi, Frankliniella borinquen*

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O301TU13

Ecology

O301

Aquatic insect assemblages in artificial ponds newly created in a Satoyama coppice in southern Osaka, central Japan

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Many species of aquatic insects are threatened due to degradation of the habitats in Satoyama, the traditional rural landscape in Japan. Recently creating artificial ponds has been made as a practice for their conservation in Japan. In this study, we placed two plastic artificial ponds (134 × 74 cm, 20 cm in depth) each inside (Site A), on the edge (Site B) of, and outside (Site C) a Satoyama coppice in southern Osaka, central Japan, and aquatic insects were counted twice a month from April to December 2011. As a result, a total of 25 species from 16 families were recorded from all the six ponds. Species richness was higher at Site A (17 species) than at Sites B and C (13), while the number of individuals was the largest at Site C (34,374), followed by Site A (19,775), and the smallest at Site B (18,178) . At Site A, Cloeon spp. (Baetidae) were dominant with the rate of occupancy > 86%, while Chironomidae spp. dominated at Sites B (> 50%) and C (> 80%). According to the cluster analyses using similarity indices, two groups of assemblages, those of Site A and Sites B and C, were recognized. Throughout the survey period, the rate of sky view was higher at Site A (47-59%) than at Sites B and C (18-43%). The results show that the structure of the aquatic insect assemblage developing in the artificial pond is strongly affected by the light condition.

Keywords:*mesocosm, succession, canopy openness, biotope, conservation*

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O301TU14

Ecology

O301

Interspecific relationships in grassland ground beetle (Coleoptera: Carabidae) assemblages

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Although interspecific relationships can significantly alter the composition of insect assemblages they are often ignored when assessing impacts of environmental change. Long-term ground beetle data were used in this study to analyse ecological networks at two sites in Scotland. Bayesian Network inference algorithm was used in order to reveal interspecific relationships. 1000 permutations of greedy searches were computed to identify possible networks. Highly probable links between nodes (species) were filtered from the permutations and the strength of each relationship was estimated. Most interspecific relationships were found to be positive. The few negative relationships had low influence scores. Pterostichus strenuus, P.nigrita and Clivina fossor appeared to have the most connections to other species, whilst Synuchus vivalis, Carabus violaceus and P. madidus appeared to be the key species within the assemblage, having strong links to several other species that persisted across replicates. One of the weak negative relationships found in the network was between Loricera pilicornis and Leistus terminatus suggesting existence of competition for their collambolan prey. Despite their high relative abundance Nebria brevicollis and Carabus problematicus did not have numerous strong links to other species. Several species had no or only one link. These species are found in high abundance in areas adjacent to the study site but rarely within the specifically studied habitat, suggesting that they do not play an important role in this particular ecological network. In contrast, species with several strong relationships are likely to be key species thus being of special interest for conservation and land management.

Keywords:*ecological network, bayesian network, Carabidae, ground beetles*

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O301TU15

Ecology

O301

The Argentine ant, *Linepithema humile*, interferes with the foraging success of native ant species in a biodiversity hotspot.

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In the Cape Floristic Region (Fynbos) of South Africa, a biodiversity hotspot characterised by high levels of endemism, Argentine ants displace myrmecochorous native ant species. However, no studies have investigated the mechanisms by which these native species are displaced. We investigated the response of Fynbos native ant species to invasion by Argentine ants by measuring their competitive ability and foraging success under laboratory conditions. Resource exploitation by native ant species and Argentine ants of equal colony sizes were compared. Argentine ants recruited the most number of workers and retrieved significantly more of the bait compared to all native species. We then compared interference and resource exploitation by a colony of native species with that of Argentine ants. During resource competition, Argentine ants did not locate the bait faster than native ants, however, once located, they rapidly displaced native ant species within the first 10 minutes of the trial through high interspecific aggression which contributed to their dominance at the bait. Argentine ants outcompeted native ants from different subfamilies and functional groups, through numerical and behavioural dominance at the bait. Pheidole capensis and Anoplolepis custodiens, thought to limit Argentine ant invasion within the Fynbos, were severely affected by Argentine ants. Their inability to compete with Argentine ants paints a bleak picture for the future of 53% of Proteacea species and 20% of Fynbos plants that depend on ants for seed dispersal. This study shows no support for biotic resistance to Argentine ant invasion in the Fynbos.

Keywords:*Argentine ants, Cape Floristic Region, Biotic resistance, Interference and exploitative competition*

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O301TU16

Ecology

O301

Diversity in the genome of *Ephestia cautella* Walker (Lepidoptera: Pyralidae) strains from Southwestern Nigeria

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This study aimed at understanding the diversity of the strains of E. cautella collected from Southwestern Nigeria. Collections of male and female strains of E. cautella were made in 16 different locations in Southwestern Nigeria. Genomic DNA was extracted from each of the sexes and the whole genome profile of each was generated using two specific primers: Mariner Transposase Gene (MTG) and Mariner Transposase Pseudogene (MTP). The primers differed in their probing capacity; while MTG identified an average of 983 total genomic length, MTP identified 977. The average gap within the length was 321.6 and 419.7 for the two primers respectively. The average Gower genetic distance which existed between the 32 strains for each primer were 0.395 and 0.407 respectively. The least (0.228, between Iyin Ekiti Male and Ile- Oluji Male) and the highest (0.476, between Iyin Ekiti Male and Awe Male) Strains genetic distances in this study occurred in Primer 1 and among the male strains. The average genetic distance among the female strains were 0.403 and 0.418 for MTG and MTP respectively. The intra-specific similarity in the studied population is about 60%, genetic variation were more significant among the male than the female strains. While results from this study unraveled the diversity within this species, it could also form a nucleus for understanding the virulence of the different strains and such could equally guide in the development of a good strategy for the control of the pest.

Keywords:*E. cautella, diversity, strains, primers, genetic distance*

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O301TU17

Ecology

O301

Species richness of canola aphids, (*Brevicoryne brassicae, Myzus persicae* and *Lipaphis erysimi*) (Homoptera: Aphididae) and its associated natural enemies in different areas of Punjab, Pakistan.

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Canola brassica napus is subject to attacked by various invertebrate pest all over the world. At least 30 species of invertebrates' pest have been found feeding on canola plants. However complex of pest species varies with crop growth stages and production area. Aphids are among most destructive pests of canola crop,its occurrence and severity of infestation have been recorded to be inconsistent during different crop seasons.Population studies of insect are one of important part of pest management strategy Studies regarding the determination of species richness of Brevicoryne brassicae, Myzus persicae and Lipaphis erysimi) (Homoptera: Aphididae).) and its associated natural enemies were conducted in different areas of Punjab, Pakistan. The objective of the study was to develop insect pest management strategy by exploring seasonal abundance of aphids at Faisalabad, Bahawalpur and Dera Ghazi Khan and Khanpur areas of Punjab, Pakistan. Top 10 cm inflorescence of six randomly selected plants in each plot was beaten 10 times with a 15 cm long stick of pencil thickness and dislodged aphids species were counted on a white paper sheet held under inflorescence. Sampling for aphid was done weekly. The specie evenness and specie richness was determined by using shanan,s index

Keywords:*population distribution, aphids, canola, natural enemies*

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O301TU18

Ecology

O301

Decrease of ant diversity due to forest disturbance

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Ant monitoring had been carried out at the LTER site in the Gwangneung forest in South Korea for 3 years from 2003. Ant diversity was significantly lower in 2004 than in 2003 and 2005. When abundance of ant species was compared among these years, forest species decreased but grassland species increased. This abrupt change was caused by forest disturbance, which was indicated by decrease of leaf area index (LAI) showing the dropping of many branches. Four functional guilds of ants differently responded to this forest disturbance; forest ground forager (FGF) and soil and litter forager (SLF) decreased but grassland ground forager (GGF) and understory vegetation forager (UVF) increased. The species richness was mainly influenced by decrease of SLF, and the species diversity (H') mainly decreased due to a sudden increase of a GGF species, Formica japonica. Through examination on weather data and insect pest, it was supposed that the forest disturbance would be caused by insect outbreak, and/or by heavy rain and/or by typhoon. Based on the present study, a mechanism was supposed for the decrease of ant diversity after the forest disturbance.

Keywords:*Ant, Climate change, Insect outbreak, Typhoon, Heavy rainfall*

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O301TU19

Ecology

O301

Altitudinal pattern of ant diversity in Korea: An empirical test of mid-domain effect and Rapoport's rule, and an alternative suggestion

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The mid-domain effect and the Rapoport' s rescue effect are diversity theories that explain prevalent diversity patterns, including hump-shaped patterns or monotonic decreases along altitude or latitude gradients. We tested whether patterns of ant species richness along altitudes in South Korea can be described by these patterns. Ants on 12 high mountains (> 1,100 m) throughout South Korea (from 33o N to 38o N) were surveyed using pitfall traps at intervals of 200 to 300 m of altitude. Abundance of ant species was significantly related with altitude and temperature. Species richness of ants decreased monotonically along altitudinal and temperature gradients. However, species richness of cold adapted species (highland species) showed a hump-shaped pattern along altitude and temperature gradients. The altitudinal and temperature ranges of ant species follow Rapoport's rule. Because of the limited occurrence of highland species in highlands, the monotonic decrease could not be explained by Rapoport's rescue effect. Based on the temperature-dependent distribution, we devised an integrative diversity hypothesis that explains local diversity patterns (monotonic decrease, hump-shaped, and monotonic increase) and global diversity patterns (peaks in the tropical region and gradually decreases toward the poles). This hypothesis predicts the prevalence of the hump-shaped pattern in warm regions, which is supported by published insect data.

Keywords:*diversity, abundance, distribution, evolution, temperature, cold tolerance*

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O301TU20

Ecology

O301

Study on settled insect communities from restored stream Cheonggyecheon, Seoul

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Last half century we've achieved economic success by development and urbanization, but it had been ignored so far that environmental destroying. After entered 21st century, however, now it is growing steadily people's interest on improving 'quality of life' but also living and natural environment. Therefore, The Seoul City Government had accomplished Cheonggyecheon Restoration Project. This unprecedented large scale restoration suggests the possibility of fulfill the harmony between human and natural environment. Especially Cheonggyecheon is now attention as the urban ecology stream, there are many insect communities which have high environmental adaptability exist with the other living organisms. In this study, we focused on those insect's settlement. We've monitored totally 18 times include day and night survey, during 2007 to 2010. The collecting results of each year are as follows: 174 species, 67 families belonging to 11 orders were recorded in the year 2007; 192 species, 70 families belonging to 10 orders were recorded in the year 2008; 206 species, 72 families belonging to 11 orders were recorded in the year 2009; 248 species, 94 families belonging to 12 orders were recorded in the year 2010; total 433 species, 125 families belonging to 13 orders were recorded. Fragmentarily this result showed that biodiversity of insect was continuously increased after restored. The important species for monitoring of Cheonggyecheon as follows: Locusta migratoria (Protected species in Seoul), Lycorma delicatula (Alien insect), Sericinus montela koreanus and Papilio xuthus (Papilionidae).

Keywords:*Cheonggyecheon, insect fauna in Seoul, restored stream, Korea, Seoul*

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O301TU21

Ecology

O301

Diversity and abundance of mesostigmata mites in Arignar Anna zoological park, Chennai, South India

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The mites comprise a large group of Arthropods belonging to subclass Acari of class Arachnida. Mites belonging to the Order Mesostigmata have gained the attention of the scientists being the potential predators of other harmful mites it's belonging to the Parasitiformes. The present study reveals the Mesostigmata species composition of mites in Arignar Anna Zoological Park (AAZP), the study was conducted during April 2011 to March 2011. The samples were collected from the cages of mammals, birds and reptiles and were examined. Mites were extracted using the Berlese method and mounted in Hoyer's medium. We encountered 23 Species belonging to Mesostigmata Orders, Species richness is dominant in Phytoseiidae family with more species abundance in genus Amblyseiinae and Macrocheles.

Keywords:*Mesostigmata Mites, Arignar Anna Zoological Park, Species richness, Shannon Wiener and Simpson index*

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O301TU22

Ecology

O301

Diversity and foraging ecology of pollinators in mango (*Mangifera indica*): An Indian perspective

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Mango (Mangifera indica L.), is the most important tropical fruit crop of India. As mango is an entomophilous crop, pollinators have a significant role to play in enhancing the productivity. Studies were conducted at Indian Institute of Horticultural Research, Bangalore, India during 2010-12 to document the diversity of insect pollinators and their foraging behavior in relation to climate as well as genotype variability. Pollinator activity during off-season on the existing vegetation in the orchard was also recorded to assess the benefit of conserving fauna during off-season. Four hundred panicles per variety were observed for pollinator density and abundance on fourteen commercially cultivated varieties. Four species viz. Apis florea (Hymenoptera: Apidae), Chrysomya megacephala and Stomorhina discolor (Diptera:Calliphoridae), Eristalinus arvorum (Dipteral: Syrphidae) were the dominant foragers significantly contributing to pollination. There were no significant differences among varieties with respect to species complex but significantly differed in terms of abundance. Indian rock bee, Apis dorsata was actively foraging in the northern part of country, representing sub-tropical climate while it was absent in other regions. Stingless bee, Trigona iridipennis was abundant in the western region characterised by coastal climate. Flowering was the single most important factor influencing the pollinator density followed by maximum temperature and relative humidity. Honey bees and dipterans responded differently to raising temperatures. Pollinators' response to climate variables is discussed which in turn would help in understanding the climate change driven shifts in mango, a highly seasonal pollinator dependent tropical fruit crop.

Keywords:*Mango, pollinator, Apis, climate change*

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O301TU23

Ecology

O301

Diversity and distribution of Chironominae (Chironomidae:Diptera) Larvae from Balaghat ranges in Maharashtra, India.

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ABSTRACT The present communication deals with the studies on diversity and distribution of larval Chironomids from Balaghat ranges of Maharashtra India. In present study subfamily Chironominae is described with twenty one species belonging to seven genera. Genera Chirnomus, Cryptochironomus, Cryptotendipes, Dicrotendipes, Glyptotendipes, Kiefferulus and Polypedilum are found in study area. Genus Chironomus and Dicrotendipes showed high diversity with five species each. Genus Polypedilum is described with four species followed by it genus Kiefferulus with three species. Genus Glyptotendipes is reprsented by two species whereas genera Cryptochironomus and Cryptotendipes are reprsented by single species each.

Keywords:*Chironomidae larvae, Chironominae, Balaghata ranges, Maharashtra.*

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O301TU24

Ecology

O301

Effect of Anthropogenic activity on diversity of insects in Nilgiri biophere Reserve, Tamil Nadu, South India

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Insects are the most diverse components of ecosystem. Monitoring of insects provide indications of the early warning of the ecological changes. In the present study we have examined the distribution and abundance of insects across the three vegetation areas of Nilgiri Biosphere Reserve. The site I represents grassland and cattle grazing areas, Site II represents the monoculture plantation of eucalyptus and site III as agricultural land. The insect diversity in Nilgiri Biosphere Reserve was investigated using pitfall trap, hand picking method, sticky glove methods, and yellow pan trapping methods from April 2010 to August 2010 which resulted in 11 orders of insects namely Lepidoptera, Odonata, Hymenoptera, Hemiptera, orthoptera, Diptera, Phasmida, Coleoptera, Dictyoptera, Isoptera, Dermaptera and 2,692 insects belonging to different orders were recorded. The diversity was calculated by using Shannon Wiener Index, resulted 2.37, 2.30, and 2.36 in site I, II and III, respectively. During the study period we recorded less abundance of insects in monoculture plantation due to the influence of human activities in the form of solid waste dumping than other sites. The insect abundance belonging to Diptera (140) and Dictyoptera (108) were dominant which adapted to Monoculture plantations. So, the present research observed the fluctuations in insect population among the sites.

Keywords:*Human activity, Nilgiri Biosphere Reserve, Shannon Wiener Index*

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O301TU25

Ecology

O301

Diversity and habitat utilization of Indian peafowl in and around Bharathiar university campus and V.O.C Park, Tamilnadu, South India

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The Pavo cristatus is Indian Peacock or Blue Peacock, called (common Peafowl) belongs to the Kingdom Animalia. It is now reported to be discontinuously distributed in its former distribution range. The present study focuses on the diversity and behavior of Pavo cristatus in Bharathair university campus and VOC park. Direct method and indirect method were carried out for the survey in selected sites. The distribution of peafowl diversity was recorded based on the survey and the same has been calculated using Shannon wiener index. The present study reveals that the diversity of peafowl's is more in domesticated than wild and captive habitat, and in domesticated the diversity of Peahen shows much abundance than Peacock. We determine the behavior of the peafowl's also shows higher variation in domesticated habitat shows the habitat is well utilized by the peafowl's inside the university campus. Compared to wild and captive the death rate and disease attack is less in domesticated habitat.

Keywords:*Pavo cristatus, Diversity, Habitat Utilization, Shannon wiener index*

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O302TU01

Ecology

O302

Effects of temperature stress on tropical insects: A case study with the brown planthopper *Nilaparvata lugens* (Stal)

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Nilaparvata lugens (Stal) is the most serious rice pest and widely distributed across Asia. Experiments were carried out to determine the effects of heat stress on nymphs and adults of N. lugens by measuring critical thermal maximum (CTmax), heat coma temperature (HCT) and upper lethal temperature (ULT50). Further experiments investigated the ability of the insect to acclimate at low (15°C) and high temperatures (30oC) compared with the standard rearing regime (23°) by determining critical thermal minimum (CTmin), CTmax, chill coma temperature (CCT), HCT, lower lethal temperature (LLT50) and ULT50. Nilaparvata lugens also showed changes in their thermal tolerance after acclimation. When the rearing temperature was decreased from 23 to 15oC the LLT50 decreased from 2.3±0.4 to 0.5 ±0.4°C in nymphs and from 0.9±0.5 to -2.7±0.4°C for adults. The CTmin was similarly decreased from 15.3±0.3 to 12.5±0.3, 13.1±0.4 to 8.1±0.2 and 12.9±0.4 to 8.8±0.2°C; and the CCT from 6.7±0.2 to 6.2±0.2, 2.1±0.2 to -1.2±0.3 and 2.6±0.2 to -0.3±0.2°C for nymphs and adult females and males. With high temperature acclimation at 30°C the ULT50 increased from 41.8±0.1 to 42.9±0.1 and 42.5±0.1 to 43.6±0.1°C for nymphs and adults, the CTmax from 34.9±1.3 to 37.2±0.1, 37.0±1.0 to 37.3±0.1 and 37.4±0.7 to 37.7±0.1 and the HCT from 37.7±1.3 to 41.0±0.3, 43.4±1.7 to 45.0±0.1 and 42.0±1.8 to 43.2±0.5°C for nymphs and adult females and males. The results indicate that nymphs of N. lugens are currently living at temperatures close to their upper thermal limits, and that acclimation ability is greater at low than at high temperature.

Keywords:*Nilaparvata lugens (Stal), temperature stress, acclimation ability, thermal tolerance*

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O302TU02

Ecology

O302

Environment driven changes in foraging behaviour of hyperparasitoid wasps and their impacts upon the structure of host-parasitoid networks

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In networks including herbivorous insects, top-down pressures predominantly drive community structure, so the foraging behaviours of parasitoid insects hold particular import for ecology (Morris et al., 2005). As occurs in predator-prey networks, we believe parasitoid foraging behaviour determines the distribution of strong and weak links within networks, which are important components of stability and structure (Brose et al., 2010). The effect of climate on parasitoid physiology is well documented, but there has been little consideration of how the external environment alters their behaviour; especially at a network level. Using a multi-year, quantified network, unique among entomological studies, we show that the hyperparasitoid wasp, Asaphes vulgaris, exerts greater parasitism pressure on populations of larger hosts compared with smaller hosts. A pattern thought to develop mainly from the host-choice decisions of the hyperparasitoids, and which corroborates the hypotheses of a previous optimal foraging framework (Henri & van Veen, 2011). The same framework predicts that the priorities of foraging parasitoids, and thereby link distribution are dependent upon external conditions, such as climate. Preliminary results of laboratory studies do indeed suggest that hyperparasitoids that are more constrained in their foraging time are less choosy. Our results suggest that climate change may have further implications for host-parasitoid dynamics beyond the obvious effects of changing physiology.

Keywords:*Host-parasitoid interactions, Climate change, Foraging behaviour, Size-structured interactions*

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O302TU03

Ecology

O302

Assessing insect responses to climate change: How are the fingerprints being detected?

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Understanding how researchers are tackling globally important issues, such as climate change, is crucial to identify whether current research is comprehensive enough to make substantive predictions about general responses to climate change. We assessed the type of studies being conducted by researchers to understand the impacts of climate change on insects, published up to June 2011. Most published research is generated from Europe and North America and being dedicated to core data analysis, with reviews being highly produced. Temperature is the main climate change driver analysed, and most researchers are assessing changes in abundance or distribution/range shifts. Highly cited studies (more than 50 citations) carry out different types of research compared to studies with less than 50 citations. Of most concern is the lack of studies focused on Hemimetabolous insect orders (which undergo gradual metamorphosis), the number of studies which do not specifically identify any climatic drivers and the need for more studies to assess specific mechanistic responses to climate change.

Keywords:*Review, desktop study, published research, citation analysis, entomology*

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O302TU04

Ecology

O302

Bark beetles and fungi: Effects of climate change on symbiont community and stability

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Many bark beetles are involved in intimate symbioses with fungi. In some cases, the fungi contribute to beetle fitness by supplementing a nutritionally poor diet. These symbioses often involve more than one mutualistic fungal partner. These partners differ in terms of the degree of benefit they confer to the host. Thus, the relative abundance of each fungus in a population at a particular time should act to negatively or positively affect beetle dynamics depending upon which symbiont dominates. The relative abundance of the various fungal symbionts with their host beetles is strongly affected by environmental conditions, particularly temperature. This sensitivity to the environment suggests that the stability of current symbiotic partnerships will be affected as climate warms. Both beetles and fungi are ectotherms, and as such, are highly sensitive to changes in temperature. However, each organism will respond in an idiosyncratic fashion. When environmental tolerances of partners cease to overlap in a way that supports continued interaction, the symbiosis will destabilize. Such destabilization not only will affect the beetle's interactions with its environment at the microbial level, but likely also at the landscape level, and ultimately influence range expansions and contractions as a changing climate alters environmental suitability. We investigated the stability of the mutualism between the mountain pine beetle and its fungal symbionts under shifting climate scenarios. Several models were developed to illuminate the historic basis for this symbiosis and help predict its geographic and temporal viability under climate change.

Keywords:*Symbiosis, bark beetle, climate change, fungi*

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O302TU05

Ecology

O302

Insect herbivory on eucalypts under changing resource allocations and climate change

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Eucalypts are associated with diverse arthropod communities, including herbivorous insects that can cause severe defoliation in plantations, native woodlands and forests. Understanding the drivers for herbivorous insect populations is required to predict adaptation and design mitigation strategies that minimise future pest outbreaks and negative impacts of climate change on forest biodiversity. We utilise the Hawkesbury Forest Experiment, a climate change field experiment on eucalypts, and a series of glasshouse experiments to test responses of insect herbivores to changes of atmospheric CO2 concentrations, temperature, water availability and plant nutrition. Our results show that physiological and developmental responses of individual herbivore species to changes of a single environmental factor such as elevated CO2 are strong, yet interactions of elevated CO2 and temperature can partially mitigate the strength of these responses. On a community level though, measurement of bottom-up versus top-down regulation of herbivory on our experimental site has revealed a strong top-down control through herbivore antagonists. Any insect herbivore response and adaptation to climate change factors may in the end be strongly shaped by the higher trophic levels that so far are the least known component within Australian eucalypt communities.

Keywords:*Eucalyptus, herbivory, climate change*

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O302TU06

Ecology

O302

Effect of climatic conditions on population dynamics of dengue mosquitoes (*Aedes aegypti* and *Aedes albopictus*).

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This study was carried out from 2009-2011 to know the effect of changing climatic conditions on population dynamics of Aedes aegypti and Aedes albopictus. Mosquitoes (larvae and pupae) were collected from fresh water ponds, sewage water ponds, fish ponds, rice fields, rain water ponds (rain gutters), tyres, tree holes, small water containers and manmade storage containers (drums, tanks, jars, buckets) in and around the homes present. Collection was done twice during every winter (October-February), summer (March-June) and moon soon season (July-September) from marked rural areas (fields, villages, towns) and urban areas within the area of 1 km2 of the Punjab. Immature stages were collected with dippers and adults with suckers. It was found that years, months of the year, seasons, temperature and relative humidity were important in case of population dynamics of mosquitoes. More number of mosquitoes was collected during 2010 and 2011 than other years due to heavy rains. High population (63 %) was recorded during rainy season due to high temperature (28-36 °C) and high relative humidity (up to 75 %) and very low or zero population was recorded from outside during winter due to low temperature (< 10° C) and low relative humidity (< 23 %), then the adults shifted in the rooms where they survived almost all the winter. A. albopictus was mostly recorded from rural areas but in parks of cities also found. A. aegypti was largely recorded from tyres, urban areas mostly during rainy season from small water containers.

Keywords:*Population dynamics, climatic conditions, dengue mosquito*

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O302TU07

Ecology

O302

Effect of climate change on seasonal foraging activity of termites (Isoptera)

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Seasonal foraging activity of termites depends principally on air tempera- tures which are increasing in recent years due to global warming. To examine the seasonal foraging activity of termites, a walking belt transects of 1 m wide was randomly laid every two weeks in four plots. Inside the frame of each plot, 100 wood logs were examined for the presence or absence of termites during one year. To determine the effect of temperature on termite foraging activity, the relation between the monthly mean temperatures (maximum and minimum) and the termite foraging activity were analyzed. The effect of climate change on this activity was analyzed by comparing our results with a previous study. In our study period, termites foraged throughout all the year. Their activities were low (but occurred) in winter. Foraging increased significantly in summer, reaching a maximum in autumn (especially for Od- ontoterms formosanus). Odontotermes species were the most active termites in our study sites. The species of this genus attacked 23% of the examined wood logs. Odontotermes dimorphus was also recorded for the first time in Hangzhou. The foraging activity of termites was positively correlated with air temperature. The occurrence of termite activity in winter and the presence of O. dimorphus in our study sites could be a result of global warming. We suggest that any management strategy against termites in these sites should be carried out at the period of high foraging activity.

Keywords:*Subterranean termites, foraging behavior, foraging activity, air temperature, belt transect, global*

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O303W01

Ecology

O303

The genomic response of grapevine to a phloem-feeding insect

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Plants respond to insect feeding damage by regulating genes that allow the plant to defend itself in ways that ultimately limit the effects of the damage. We examined the interaction, at genomic level, between grapevine Vitis vinifera L. and a phloem-feeding insect pest, the vine mealybug Planococcus ficus (Homoptera: Pseudococcidae). Mealybugs were allowed to feed on grapevine for periods of 6 and 96 hours, after which plant transcripts were examined using microarray analysis with a 135k NimbleGen gene expression grape array. Grapevine response to mealybug feeding at intermediate periods of mealybug feeding (24, 48 and 72 hours) was investigated using qPCR analysis of ten genes known to be associated with plant defence against insects. After 6 hours of mealybug feeding, microarray analysis showed that only 107 transcripts were differentially expressed in grapevine plants. This number increased slightly to 149 transcripts after 96 hours of mealybug feeding. qPCR analysis showed that none of the genes analysed were differentially regulated after 24 and 72 hours of feeding. However, after 48 hours of mealybug feeding, a single gene, pathogenesis-related protein 1, was differentially expressed compared to control plants. Thus, both microarray and qPCR analysis showed that grapevine response to mealybug feeding was weak. In addition, microarray analysis determined that many of the genes affected by mealybug feeding are also affected by microorganism attack. This pattern indicates similar modes of evolution of resistance mechanisms against a variety of biotrophs.

Keywords:*genomics, grapevine, mealybug, microarray, qPCR*

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O303W02

Ecology

O303

Does the wooly poplar aphid, *Phloeomyzus passerinii*, manipulate the defense reactions of its host tree?

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The wooly poplar aphid is a major pest of poplar stands in Southern Europe, the Near East and Northern Africa. After both intercellular and intracellular stylet pathway, it feeds exclusively in the cortical parenchyma of poplar trunks and, during outbreaks, this aphid causes extended cortical necroses and branches drying that can lead to tree death. We have compared the cortical reaction to both mechanical wounding and aphid's feeding of three Populus x canadensis genotypes with contrasted resistance levels (i.e. 'I214' which is susceptible, 'I45/51' which affects aphids' demographic parameters and 'Brenta' which impedes aphids' development). Each week during one month, we performed histological sections around stylet insertion sites to compare anatomical and biochemical modifications of bark tissues. In parallel, we have investigated how these reactions affected aphid's demographic parameters by comparing P. passerinii's development between pre-infested and control plants. Mechanical wounds induced localized wound periderms while aphid feeding triggered extended reactions, sharing similarities with a wound periderm, but exhibiting also thin-walled hypertrophied cells and typical lignins, tannins and flavanols accumulation patterns. Vacuolar phenolic compounds and lignins were more concentrated in resistant genotypes' reactions. Aphids developing on these reactions zones, after a pre-infestation, exhibited a higher rate of natural increase compared to aphids developing on uninfested host tissues. We hypothesize that P. passerinii, with its destructive feeding mode, triggers poplars defense mechanisms and manipulates them to initiate the formation of a pseudogall.

Keywords:*resistance, phenolic compounds, lignification, wound periderm, gall*

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O303W03

Ecology

O303

The effects of wind and bees on Pollen-mediated gene flow from transgenic *Bt* cotton

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Development and commercialization of insect-resistant genetically modified (IRGM) crops expressing insecticidal proteins (Cry toxins) from bacterium Bacillus thuringiensis (Bt) have offered an alternative to traditional synthetic insecticides for control of important agricultural insect pests. Considerable discussion was addressed about the risk assessment of transgenic plants. In this study, pollen-mediated gene flow (PGF) from the transgenic Bt cotton was tested in the greenhouse. Based on PCR and protein detection, we compared the distance and the frequency of gene escape between the two treatments with wind and with bees. The results showed that the frequency of gene flow increased significantly in both wind and bees treatments compared with the treatment without wind and bees. In detection range, the maximal gene flow distance observed was 36m in both wind and bee treatments. PGF of the island cotton was higher under the condition with inconstant wind direction than that with the constant one. The rate of Bt gene expression in bee treatment was higher than that in wind treatment. These results suggest that pollen-mediated gene dispersal of cotton could enable widespread gene flow between transgenic crops and conventional varieties under current farming practice.

Keywords:*transgenic cotton, Bt gene, gene dispersal, pollen-mediated, biosafety*

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O303W04

Ecology

O303

Distribution of *Pentalonia nigronervosa, P. caladii*, and the Banana Bunchy-Top Virus in Micronesia

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A survey of the distribution of Pentalonia nigronervosa and P. caladii, and the incidence rate of the banana bunchy top virus (BBTV) was conducted in Micronesia. Aphids and plant tissues were collected from banana and non-banana host plants in the Micronesian islands. All plant samples were tested for the presence or absence of BBTV using an enzyme linked immunosorbent assay (ELISA). Concurrently, host-switching experiments were conducted to determine the ability of the Pentalonia aphids to successfully sustain colonies upon switching from one host to another. ELISA analysis showed that BBTV is present on Guam, Saipan, Tinian, and Rota but is absent elsewhere in Micronesia. Pentalonia aphids were identified to species level using morphometric and genetic analysis. Host-switching experiments are on-going, but early trials show little successful transfers. P. nigronervosa, collected on Musa sp., occasionally transfers onto Alpinia purpurata. P. nigronervosa, collected on Musa sp., rarely transfers onto Heliconia stricta. P. caladii, collected on A. purpurata, occasionally transfers onto Musa sp. P. caladii, collected on H. stricta, rarely transfers onto Musa sp. These results of the early trials suggest that Pentalonia colonies cannot successfully maintain populations on non-host plants.

Keywords:*Banana Bunchy-Top Virus, Pentalonia nigronervosa, Pentalonia caladii*

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O303W05

Ecology

O303

Optimal defense strategy in an extrafloral-nectar bearing plant using physical, chemical and biotic defense traits

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Extrafloral-nectar (EFN) bearing plants employ diverse defense tactics. Many previous studies have demonstrated that physical and chemical defenses are effective to various herbivores. However, following questions have not yet been answered: why do plants entrust defense to ant workers and how do they utilize multiple defense traits? An EFN bearing plant Mallotus japonicus possesses trichome, pellucid dot, and EFN and pearl body as physical, chemical, and biotic defense traits, respectively. In this study, we estimated the production cost of each defense trait. As a result, the increase of trichome and pellucid dot densities reduced the plant growth, whereas that of EFN and pearl body numbers did not. This indicates that the production costs of EFN and pearl body are lower than those of trichome and pellucid dot. Then we examined the effectiveness of each defense tactics. On newly extended leaves, trichome and pellucid dot densities were extremely high, but they decreased rapidly with leaf age. A laboratory bioassay revealed that trichomes and pellucid dots in young leaves acted well against herbivores, but those in middle-aged leaves did not. EFNs and pearl bodies attracted more ants on middle-aged leaves than on other leaves, but some M. japonicus plants were found to carry no ants, suggesting that the biotic defence using ants is less stable than physical and chemical defenses. The shift from the physical and chemical defenses to biotic defense with leaf age is considered to maximize the fitness of M. japonicus.

Keywords:*ant-plant interaction, plant defense, multiple traits, Geometridae, leaf age*

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O303W06

Ecology

O303

Battus philenor larvae use their frontal projections as host plant detectors

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Some butterfly larvae have a pair of long projections in their frontal parts which are different from antennas. However, the role of these projections has yet to be tested. The larvae of Pipevine Swallowtail butterfly, Battus philenor, also have a pair of long fleshy frontal projections and the larvae can actively move the projections up and down. Here we hypothesized that these larvae use their frontal projections as host plant detectors while they search for host plants and tested this hypothesis. Field experiments in Santa Rita, Arizona, USA revealed that the individual plants of Aristolochia watsonii, the main host plant around this area, are rather small and scattered, that the larvae often need more than one host plant to complete their development, and therefore they have to move around to find new individual host plant after eating all of the previous individual host plant. Laboratory experiment revealed that host-plant discovery rate during searching for host plants were significantly higher for normal larvae than for the larvae whose frontal projections were cut. The visual information via lateral ocelli also contributed to increase discovery rate. To the best of our knowledge, this is the first evidence demonstrating that projections, other than legs or antennas, plays an important role of finding host plants in insects.

Keywords:*long fleshy frontal projections, Pipevine swallowtail, Aristolochia watsonii, Searching for host plants*

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O303W07

Ecology

O303

Gall biology of an Eulophid wasp species associated to flower ovaries of *Philodendron bipinnatifidum* (Araceae) in Brazil

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We studied between 2010-2012 the galling biology of a tetrastichine wasp (Hymenoptera: Eulophidae) that develops in inflorescences of Philodendron bipinnatifidum (Araceae). We assigned to individual inflorescences of six plants the following treatments: oviposited-pollinated (O-P), oviposited-unpollinated (O-U), not oviposited-pollinated (N-P) and Control (C). Inflorescences were protected by tissue bags to prevent natural access of insects. We recorded the abortion for each treated inflorescence and quantified the number of galled flowers/fruits and fruits with seeds. For the galling process, we introduced 30 wasps in bagged inflorescences and let them oviposit for 48h. From oviposition day we collected each 5 days 5-10 galled flowers. We destined galls to manual dissection and histology. Absence of pollination seemed to favor galling capacity. Percentage of abortion per treatment was 100% (C, n=25), 33% (O-P, n=15), 64% (O-U, n=28) and 33% (N-P, n=24). Total flowers per inflorescences was 290±112 (n=26). Number of galled flowers was 202±69 (O-U, n=7), 129±122 (O-P, n=12). Number of fruits with seeds was 280±132 (N-P, n=7) and 171±117 (O-P, n=13). Wasp egg is deposited inside the ovary locule. First larval stage (0.31±0.10mm, n=60; 5-15 days) kept egg location. Simultaneous to gall formation, second larval stage (1.19±0.47mm, n=40; 20-30 days) began to chew the integuments and established at the embryo sac cavity where it grew and achieved the final third stage (2.41±0.56mm, n=40; 35-40 days). This is the first record describing the galling biology for the group. Financial support: S.J.G. FAPESP#09/10237-9. R.A.S.P. FAPESP#04/10299-4 and CNPq#302769/2008-0. S.P.T. CNPq #301960/2009-7.

Keywords:*Gall formation, Larval biology, Eulophidae, Chalcodiodea, Philodendron*

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O303W08

Ecology

O303

Host selection features of xylophagous insects and their applications in forest health assessment

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Forest health diagnosis and assessment is a rising hotspot in research and development of world forestry since 1990s. On this subject, former researchers have done some research work mainly in the aspects of forest vigor, organization, anti-menace and resilience, ecological service functions etc., however, few research papers were reported about diagnosing and assessing forest health in virtue of xylophagous insects being bioindicators based on the fact that secondary xylophagous insects prefer attacking sub-healthy and unhealthy trees. In this paper, underlying rules that secondary xylophagous insects are always in accompany with sub-healthy and unhealthy trees were explored and verified in the case of a secondary xylophagous insect-Monochamus alternatus at first. Then, suitable Pinus yunnanensis virgin forests, secondary forests and forest plantations were selected as research plots in core zone and marginal zone of geographic distribution of Pinus yunnanensis in Yunnan, China. Based on natural qualities of forest ecosystem, tree health status of Pinus yunnanensis were divided into 5 grades: healthy, sub-healthy, unhealthy, dying and dead; also, harm conditions of xylophagous insects (existing species and amount) were investigated in these plots by using bait wood and insect trap. After that, forest health index(FHI)was established to quantify forest health status. Finally, correlations and regression equations were established between forest health index of Pinus yunnanensis and harm conditions of xylophagous insects. The research aims were exploring feasibility of forest health assessment in virtue of xylophagous insects being bioindicators, and providing academic and experimental bases for assessment and sustainable management of Pinus yunnanensis forest health.

Keywords:*xylophagous insect, Monochamus alternatus, forest health, Pinus yunnanensis, bioindicator*

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O303W09

Ecology

O303

Host plant manipulation by a gall-inducing insect: Regrowth response affects on herbivores abundance

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Insect herbivores manipulate physiology, phenology and nutrition of host plants, thus may effect on abundance and performance of other herbivores. We investigated regrowth effects by a chestnut gall wasp Dryocosmus kuriphilus, and its effect on other herbivore. A pair of 1-year old galled and ungalled shoots was randomly selected. Physical and nutritional changes in the host plant in terms of leaf thickness and toughness, and nutrition content were measured. Gall initiation stimulated the formation of new leaves and accelerated maturation leaves during May to August. Leaves emerged from galled shoots were tougher and thicker, and also had higher nitrogen but low water content. Colonization by the aphid Myzocallis kuricola was significantly higher on leaves emerged from galled shoots. Gall initiation positively affected the abundance of aphids by enhancing the availability of food resources as a result of the regrowth responses in the chestnut.

Keywords:*host-plant manipulation, regrowth response, gall-inducing insects, herbivores, Dryocosmus kuriphilus, Myzocallis kuricola*

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O303W10

Ecology

O303

Feeding and growth of cotton armyworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) in response to cotton varietal characteristics

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This study investigated morphological and biochemical characteristics of CB9, CB10 and SR05 cotton varieties and observed their effect on feeding and growth of cotton armyworm Spodoptera litura. Morphological characters of plant architecture, branching, color, hairiness, trichome density and boll size indicated that CB9 is a bushy, well developed, branched, light bronze colored and hairy variety with number of trichomes 193/cm2 and boll weight 5.5?6.0 g/boll. Both CB9 and CB10 bear normal leaves and spherical bolls whereas SR05 has well-ventilated branching, okra shaped and ventrally arranged light bronze colored leaves and oval bolls ranging 4.0?4.5 g/boll. Biochemical analysis indicated significant differences in starch and protein contents, which range 11.4?21.3 and 17.5?30.6%, respectively. The lowest percentages of starch and protein were found in SR05 and CB9, respectively. Cotton varieties showed significant effects on second, third, fourth and fifth instar larval weight and they attained the highest weights (22.7, 133.8, 168.2 and 1190.2 mg, respectively) when fed SR05. Larvae and moths fed SR05 also showed the significantly highest cocoon, exuviae, and adult male and female weights (434.5, 19.2, 161.3 and 232.4 mg, respectively). The cotton armyworm showed the significantly highest growth index (1.5) as well as lowest feeding index (0.05) on SR05. Results of this study indicated that SR05 is suitable for larval feeding and development, and suggest that this variety is least suitable for cotton growers in areas where S. litura is a major pest.

Keywords:*Cotton variety, morphological features, protein, starch, Spodoptera litura*

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O303W11

Ecology

O303

Routes and processes leading to New World-Old World and North America-South America disjunctions in three widespread spider wasp groups (Hymenoptera: Pompilidae)

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Ceropales, Anoplius and Aporini are three widespread groups of spider wasps. The females of these pompilids have different behaviors that range from host capturing and dragging, to the use of a host captured by another spider wasp (cleptoparasitism). Members of these groups are distributed worldwide, but the fossil record and preliminary dating analyses suggest that Pompilidae, and thus Ceropales, Anoplius and Aporini are no older than 50Ma. Because they are younger than Pangaea, Laurasia, or Gondwana, processes not related to continental drift must explain their present distribution. Our aim was to determine the age and processes that led to the cosmopolitan distribution of Ceropales, Anoplius and Aporini, and compare them. We studied the historical biogeography of these groups using molecular data to construct a chronogram, which was dated using fossils. We also estimated ancestral areas using a Bayesian approach. Our results suggest that all the groups originated in different areas in the Late Oligocene. The events leading to dispersal between the New World and Old World occurred at the same time in the Early Miocene, and the events leading to movement between North America and South America occurred between the early and middle Miocene, with some differences in timing between the groups.

Keywords:*Pompilidae, Biogeography, Molecular Systematics*

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O303W12

Ecology

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A possible seasonal trade-off between the number of dispersed seeds and long-distance seed dispersal in myrmecochorous sedges, *Carex lanceolata* and *C. tristachya* (Cyperaceae)

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Seed dispersal affects the distribution of plant individuals, and hence their fitness. Thus, clarifications of traits determining the dispersal patterns and their evolutionary mechanisms are fundamentally important. The number of dispersed seeds and seed dispersal distance, both of which are related to the dispersal success, could change seasonally with overall density and species composition of seed dispersers. Therefore, fruiting phenology should evolve to maximize the dispersal success. To test this hypothesis, seasonal changes in the overall density and species composition of dispersers must be clarified. In this study, I surveyed these two disperser attributes periodically during the fruiting periods of two myrmecochorous sedges, Carex lanceolata and C. tristachya. Then I measured seed dispersal distances by several ant species. The density of overall ants increased with date, but the proportion of Formica japonica, the largest species and a long-distance seed disperser, decreased. As a consequence, earlier fruiting of C. lanceolata synchronized with high proportion of the long-distance disperser, while later fruiting of C. tristachya did with high density of the disperser assemblage. These results indicate that interspecific differences in the fruiting period among sympatric ant-dispersed plants reflect the difference in the relative importance of the number of dispersed seeds and long distance seed dispersal.

Keywords:*Fruiting phenology, Seed dispersal by ants, seed dispersal strategies*

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O303W13

Ecology

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Genetic structure and diversity of the populations of an invasive species *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in China

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The codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae) is one of the most serious pest insects in apple orchards. This pest was firstly reported in China in the 1950s and was considered one of the most serious invasive pest in fruit orchard. It spread rapidly from the original site in Xinjiang to other northwestern regions, and has also invaded into some northeastern regions since 2006. With its rapid invasion rate, most apple growing regions of China is being threatened. As yet there has been no research into the genetic structure and genetic diversity of codling moth populations in China. In this study, we collected C. pomonella populations throughout the invested regions of China. Microsatellites and mitochondrial genes were used to investigate the genetic structure and diversity of these sampled populations. It was observed that there was significant genetic differentiation among populations. The populations from Heilongjiang, northeastern China, revealed distinct genetic structure and higher genetic diversity in comparison with populations from Xinjiang and Gansu, northwestern China. Meanwhile, populations from northern Xinjiang showed similar genetic diversity and genetic structure, whereas the two populations from southern Xinjiang also shared similar genetic diversity and structure. Furthermore, genetic diversity of the three populations from Hexi Corridor was of the same level as the population from the adjacent region of Xinjiang. It was possoble that the northerstern populations had dfferent invasive origin of the northwestern populations. Geographic isolation, human-aided dispersaql and the flight capacity of influence population genetics of codling moth populations in China.

Keywords:*Cydia pomonella, genetic diversity,genetic structure, gene flow*

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O303W14

Ecology

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Role of hoverfly - plant species interaction in maintenance of pea aphid host races

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The pea aphid (Acyrthosiphon pisum) complex consist of at least 11 genetically distinct host races which are native to specific host plants, but can all develop on the universal host plant Vicia faba. The factors that gave rise to and maintain these host races are not yet known. If natural enemies prefer certain plants for prey searching or oviposition, this might cause aphids to favour other plants (enemy free space) as hosts and thus help to maintain the host races. The attractiveness of a certain plant for natural enemies might depend on plant architecture, volatile profile and chemical composition which may alter the nutritional value and toxin content of aphids, thereby influencing natural enemy performance. We tested for the existence of enemy free space by exposing three pea aphid host races feeding either on their respective native host plants, Medicago sativa, Trifolium pratense, or Pisum sativum, or on the universal host plant to natural predator populations in the field. For each aphid host race we found a strong oviposition preference of hoverflies (Episyrphus balteatus) for the universal host plant. Since hoverfly larvae are important aphid predators we conclude that they may force pea aphids to use other hosts. In a laboratory experiment, we monitored the performance of hoverfly larvae fed with aphids reared either on the native or the universal host plant. Interestingly, larval performance was not influenced by the plant species the aphid prey was feeding on. Thus factors other than plant chemical composition may explain the oviposition pattern.

Keywords:*multitrophic interaction, enemy free space, sympatric speciation, pea aphid, hoverfly larval performance*

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Ecology

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Acquisition of plant viruses changes host plant preference of aphid vectors

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Plant virus infection can alter the suitability of host plants for their aphid vectors. Most reports indicate that virus-infected plants are superior hosts for vectors compared to noninfected plants with respect to vector growth rates, fecundity and longevity. Some aphid vectors preferentially respond to virus-infected plants compared to noninfected ones, while others avoid infected plants that are inferior hosts. Thus, it appears vectors can exploit changes in host plant quality associated with viral infection. Enhanced vector performance and preference for virus-infected plants might also be advantageous for viruses by promoting their spread. Our research has focused on two of the most important members of the virus family Luteoviridae that infect wheat, [Barley yellow dwarf virus (BYDV)], or potato, [Potato leafroll virus (PLRV)], and their respective aphid vectors, the bird-cherry oat aphid, Rhopalosiphum padi, and the green peach aphid, Myzus persicae. Our recent studies have examined preferences of viruliferous and nonviruliferous aphids for virus-infected or sham-inoculated plants (plants challenged with nonviruliferous aphids). In both pathosystems, nonviruliferous aphids significantly preferred virus-infected plants, while viruliferous aphids significantly preferred sham-inoculated plants. This shift in preference from infected to noninfected plants following virus acquisition could accelerate the rate of virus spread. Ongoing work aims to determine the mechanisms causing these changes in aphid behavior and implications for virus spread. Recent findings from our research will be presented and potential impacts on vector ecology and virus disease epidemiology will be highlighted.

Keywords:*Aphids, Virus vectors, Host plant selection behavior, Virus epidemiology, Vector ecology*

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O303W16

Ecology

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Effect of host plant species on bionomic and life history parameters of *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae) a parasitoid of the mango mealybug *Rastrococcus iceryoides* Green (Homopte)

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Anagyrus pseudococci Girault (Hymenoptera: Encyrtidae) is a solitary, koinobiont endoparasitoid of several mealybug species. The effect of five host plants (Mangifera indica L., Cucurbita moschata Duchesne, Parkinsonia aculeata L., Cajanus cajan L., and Ficus benjamina Roxb.) on host acceptability for oviposition and suitability for immature development of this parasitoid in the invasive mango mealybug Rastrococcus iceryoides Green (Homoptera: Pseudococcidae) was investigated. Effect of host plant on fitness trait (parasitoid size, egg load and longevity) and life table parameters was also assessed. Although, A. pseudococci accepted the mealybugs regardless of the host plant, the level of acceptability varied significantly. The highest percentages of parasitized nymphs were recorded on C. moschata, P. aculeata, and M. indica, while it was lowest on F. benjamina. Host suitability was also strongly affected by the host plant and largely mirrored host acceptability for all the parameters evaluated. Female wasps reared on mealybugs maintained on C. moschata and P. aculeata were biggest and more fecund, while those reared from mealybugs maintained on F. benjamina were of inferior quality with regard to all fitness parameters evaluated. Anagyrus pseudococci achieved a greater intrinsic rate of natural increase (rm), net reproductive rate (Ro), finite rate of increase (λ) in addition to a shorter mean generation time (G) and population doubling time (Td) on mealybugs maintained on C. moschata and the reverse was true for those maintained on F. benjamina. The findings are discussed in view of improvement of laboratory mass rearing, as well as field enhancement of the parasitoid performance

Keywords:*Host plants, Anagyrus pseudococci, host acceptability, host suitability, life table parameters.*

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O303W17

Ecology

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The relationship between age and learning ability of *Venturia canescens*

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Several different physiological and biological differences occur in adults while they get ageing. In most cases ageing can affect efficacy of living organism negatively and lead to decrease organism performance. V. canescens spawn in sexual and asexual way but in most cases it occurs in asexual system and all offspring will be female. Males are too rare and observed rarely. This species is considered as one of the most important parasitoids of Pyralid moths. This survey was conducted to study ageing effects on learning ability and differences in this ability on different adults' age. Thirty fifth age larvae of flour moth were introduced to boxes (20×8 cm) and 20 one day old and naive adult wasps were introduced to these boxes and were maintained in odor tanks for five hours. Peppermint odor was pumped into the tanks, and then olfactory responses of wasps were tested in Y maze olfactometer. According to our results olfactory learning ability decrease in response to adults ageing, from one day old to fourth day old, responses curve showed a decrease with a lesser extent while it decreased extremely after forth day old. According to obtained results no significant differences observed in learning ability of wasps after forth day old.

Keywords:*learning, Venturia canescens, olfactometer*

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O304TH01

Ecology

O304

Movement of parasitized aphids favour inbreeding avoidance of adult parasitoids

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In insect, mating strategies depend notably on the spatial and temporal emergence pattern of adults. In quasi-gregarious parasitoids, adults emerge from patchily distributed hosts and the rhytmicity of their emergence favour on-patch mating. Aphid parasitoids are considered to behave as quasi-gregarious species because of the patchy distribution of their hosts, but no evidence of the patchy and synchronized distribution of emergences has been proved to that date. We used a tri-trophic system composed of Aphidius matricariae (Hymenoptera, Braconidae), the aphid host Myzus persicae and turnip as host plant to investigate if this species behaves more as a solitary species or as a quasi-gregarious one because of the aphid behaviour after parasitism and the daily rhythmicity of emergences. We first observed that the immediate consequence of a female parasitoid attack on the aphid colony is aphid dispersal. As a consequence, adult parasitoids often emerge from mummies that are not distributed in patches in the environment and have to disperse to find a mate. Second, the solitary emergence of adults is reinforced by the observation of their daily rhytmicity of emergence. For a same cohort of parasitized aphids, emergences spread over 3 days and males emerge in average before females, resulting in a delay emergence of brothers and sister. We will discuss the adaptative values of both spatial and temporal emergence structures and how can it favour our understanding of the mating system and strategies of aphid parasitoids.

Keywords:*inbreeding, mating strategies, host modified behaviour*

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O304TH02
Ecology

O304

Nesting habit of *Osmia orientalis* and its parasitoids *Chrysura hirsuta*

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Osmia bees are important pollinator for crop production. Nest biology and natural enemy has been investigated in many species that nesting in woods and stems. However, little is known about the nesting habitats of Osmia bees nesting in snail shells and the parasitism by cuckoo wasps. We investigated 1) the nesting rate of Osmia orientalis Benoist and 2) the rate of parasitism and sex ratio of the cuckoo wasp Chrysura hirsuta (Gerstaecker) that only parasitizes O. orientalis in Japan. We successfully led them to nest in the empty snail shells which we placed in the field. As a nesting environment, they preferred glass fields rather than denuded, adjacent to buildings, or forest edge. The nesting rate was higher for larger shells of Euhadra amaliae than for smaller ones of Satsuma japonica. The rate was also higher for intact shells than for damaged ones. The overall sex ratio within the shell nests was rather male biased. Sex distribution within the shell nests was difference with shell species. The female ratio gradually increased towards the outer cells and became highest in the second outer cells. The percentage of parasitism by cuckoo wasps per all collected cocoons tended to be high (20-50%). From our results and previous reports, the adult sex ratio of C. hirsuta was strongly female-biased, which suggests that the species reproduces via thelytokous parthenogenesis. Further work would reveal both nesting strategy of O. orientalis and reproductive strategy of C. hirsuta.

Keywords:*Megachilidae, nest, parasitism, snail shell, sex ratio, solitary bee*

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O304TH03

Ecology

O304

Why does *Oreina cacaliae* (Coleoptera: Chrysomelidae) take the decision to fly and leave its host plant?

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In the Swiss Alps a population of the Chrysomelid Oreina cacaliae has been observed for several years, where one part of the population engages in flight in autumn and spring, whereas the other part does not fly. Here we address the question if costs are associated with the decision to fly. Factors like number of larvae produced, life span, and mating success were analyzed. We found that flyers were smaller in size and lighter in weight than non-flyers. Smaller female beetles were reduced in larvae production compared to bigger ones. We found that non-flyers lived longer and produced more larvae than flyers. Wing size analysis showed no difference between flyer and non-flyer groups. Behavioural mating experiments showed that heavy beetles (males as well as females) were more successful in (multiple) mating than lighter ones. To our surprise freshly emerged new generation females did not have higher mating success than older females. Can it be that the lighter/smaller beetles decide to leave the host plants by flight because they have a reduced fecundity and are less successful as mating partners?

Keywords:*flight, mate choice, reproduction,wing-size,*

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O304TH04

Ecology

O304

Senescence in immune priming and attractiveness in *Tenebrio molitor*

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Age-related decline in immune activity is referred to as immunosenescence and has been observed for both the adaptive immune response of vertebrates and the innate immune system of invertebrates. Since maintaining a basic level of immune defence and mounting an immune response is costly, optimal investment in immune function should vary over a wide range of individual states such as the individual's age. In this study we tested whether the immune response and immunological priming within individuals become less efficient with age using mealworm beetles, Tenebrio molitor, as a model organism. We also tested whether aging and immunological priming affected the odours produced by males. We found that young males of T.molitor were capable of mounting an immune response a sterile nylon monofilament implant with the potential to exhibit a simple form of immune memory through mechanisms of immune priming. Older males did not increase their immune response to a second immune challenge, which negatively affected their sexual attractiveness and remaining life span. Our results indicate that the immune system of older males in T.molitor is less effective, suggesting complex evolutionary trade-offs between ageing, immune response and sexual attractiveness.

Keywords:*Senescence of innate immunity, Encapsulation response, Tenebrio molitor, Sexual attractiveness, Life span*

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O304TH05

Ecology

O304

Sexual pheromones evolution in insular bumblebees

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The courtship signal allows pre-mating recognition. Its evolution depends on the sexual selective pressure mainly lead by interspecific interactions. In sympatry with Closely Related Species (CRS), the selection for specific signals to avoid mismatch make this pressure stabilizing. This leads to a saltational evolution across phylogenetic lineage. Conversely, in allopatry with CRS, as in many insular systems, the lack of hydridization risk may promote relaxation of the stabilizing selection and change the courtship signal evolution. We tested this hypothesis using genetic and chemical comparative approaches on a courtship signal trait, the Sexual Marking Pheromones (SMP) of four bumblebee species including Corsican insular (allopatry with CRS) and mainland (sympatry with CRS) populations. Analyses showed 1) genetic and SMP differentiation of insulars and, 2) a lower genetic variability and a higher SMP variability of insulars. A correlation between the neutral loci distance and the SMP distance was detected for the insulars. Differentiation of Corsican bumblebees has led to rapid speciation in ca 15 000 years. The increase of the SMP variability and the correlation genetic-SMP suggested a relaxation of the stabilizing selection on the courtship signal leading to a gradual differentiation in insular system with species free of their CRS.

Keywords:*Courtship signal, Speciation, Island*

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O304TH06

Ecology

O304

How do insects find differently sized realistic odour plumes? Model and experiment

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Chemical information is the main basis for insects to gather information about the value of their environment. The chemicals conveying information are known as infochemicals. The wind disperses odour molecules from a substrate into the environment. The structure of such an odour plume is varying over time. At a small scale the plume has an intermittent structure: patches with high odour concentrations are interspersed with regions with no odour at all. The full odour plume meanders as it moves with the wind. Here, we compare the odour concentrations of a so-called filamentous plume model that takes the features of a real odour plume into account, with measurements from experiments on detection of odours by insects. We also investigate how settlement of insects differs with the size of an odour plume and the threshold value for detection of the odours by the insects.

Keywords:*filamentous plume, Gaussian plume, spatial distribution, settlement, odour source discrimination*

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O304TH07

Ecology

O304

Effect of migration and mating status of the legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) on the efficacy of synthetic sex pheromone traps in West Africa

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Synthetic sex pheromone traps for the legume pod borer, Maruca vitrata, effectively attracted males in cowpea fields at Cotonou in southern Benin, where pest incidence is observed throughout the year. However, the same pheromone lures caught very few moths in areas north of latitude 9° N in Benin, Nigeria and Niger, despite the fact that there were a large number of catches in light trap collections. To clarify the relationship between reproductive ability and migration in M. vitrata, sex and female mating status of the individuals caught by light traps were examined. The results showed that light-trap catches were more female-biased from August to October, a peak period of the moth population, at Kandi and Kano. After October, the female-ratio decreased in Kano. In contrast, the Cotonou population was male-biased from July to August, and shifted to a female-bias from November to January. The proportion of mated females was higher at the beginning of incidence at Kano, before the mating rate decreased after September. A hypothesis was presented to explain the seasonal changes in reproduction and migration of the pest as follows: M. vitrata moths are sexually active in the south humid zone, thus they can mate. After mating, more female moths migrate from south to north, while fewer males migrate. The immigrants and their offspring are sexually inactive in the dry zone for unknown reasons. These might be part of the reason why pheromone traps are not effective for M. vitrata males in the dry savanna of West Africa.

Keywords:*Maruca vitrata, synthetic pheromone trap, light trap, migration, sex distribution, mating status, Benin, Nigeria, Niger, ITCZ*

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O304TH08

Ecology

O304

Pheromones and body coloration affect mate recognition in *Amata* genus

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Japanese nine-spotted moth Amata fortunei and related species A. germana were diurnal moths and sympatric. We observed that A. fortunei males flow in the early morning, while A. germana males did in the late afternoon. Adults have different body colorations: A. fortunei have two yellow bands on the 1st and 5th segments of their abdomens (two-band type); A. germana have yellow bands on each segment (each-band type). To evaluate the importance of visual cues in mating behavior, we conducted the experiments with crude extract and an additional model stimulant. A. fortunei males responded more to model females with the same number and a similar area of yellow bands to the original conspecific females than to those with more bands and a greater total band area, suggesting that a dissimilarity in band number and area to conspecific females could interrupt male mating behavior. On the other hand, A. germana males responded to females A. fortunei as same as conspecific females, suggesting that importance of visual cues in mating behavior of A. germana could less than that of A. fortunei. In conclusion, two species have different time schedules for mating and different sex pheromone components are involved (KonDo et al. unpublished data). These suggest that both differences in pheromone components and body coloration played a role in speciation within the genus.

Keywords:*Amata fortunei, Amata germana, Syntominae, sex pheromone, body coloration, mate recognition*

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O304TH09

Ecology

O304

Behavioral and chemical differences among females in the temperate paper wasp *Polistes snelleni*.

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Polistes wasps are primitively eusocial insects with less specialized female castes. In their societies, aggressive behaviors among females play important roles to regulate their reproductive hierarchies. In addition, cuticular hydrocarbon profiles (CHCs) reliably provide information about the fertility differences among the females. Previous studies have suggested that unmated egg-laying workers in orphaned colonies produce CHCs resembling to those of queens. It has been predicted that caste determination in Polistes wasps should be absent or occur in post-emergence period because workers seem to be totipotent. Workers of Polistes snelleni are capable of reproducing female offspring directly via fertilized eggs. We compared behaviors and CHCs of females in queenright and orphaned colonies. Egg-laying workers in orphaned colonies showed aggressive behavior more frequently than queens. In addition, there were differences in CHCs between queens and workers. Although mated egg-laying workers showed rather similar CHC profiles to queens' CHCs, those of other workers were far distinct from queens'. These results suggest that the CHC profiles of the female wasps are honest signals reflecting their fertility and caste differences. Because there were behavioral and chemical differences between queens and workers, mating status seems to be determinant effect on the females status in P. snelleni.

Keywords:*social insects, eusocial wasps, Hymenoptera, worker reproduction*

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O304TH10

Ecology

O304

Changes in apple plant volatiles in response to feeding damage of *Pandemis heparana*

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The volatile organic compounds (VOCs) emitted by the leaves of young apple seedlings of Golden Delicious were collected by close loop stripping analysis and characterized by the gas-chromatography mass-spectrometry. Volatile emissions were taken from undamaged, mechanically damaged and insect damaged seedlings. The insect damaged seedlings were previously infested with ten larvae (IV instar) of one of the major apple pests, namely the moth Pandemis heparana (Lepidoptera: Tortricidae). Volatiles were collected for three subsequent days. In the headspace of undamaged plants 12 volatile compounds were indentified, among which the green-leaf-volatiles (Z)-3-hexenyl acetate and ethyl-hexanol, together with the aldehydes nonanal and decanal. In case of mechanically damaged leaves 18 compounds were detected, where (Z)-3-hexenyl acetate represented the highest peak. Some compounds, as acrolein diethyl acetal, (E)-4,8-dimethyl-1,3,7-nonatriene, (Z)-3-hexenyl butyrate, (Z)-3-hexenyl benzoate, indole and α-farnesene were found in the headspace of mechanically damaged plants but not in healthy plants. Six hours after damage (Z)-3-hexenyl acetate concentrations strongly decreased. In the headspace of seedlings damaged by P. heparana 26 volatile compounds were characterized. Among them, some VOCs were uniquely induced, as the monoterpene (E)-β-ocimene, the terpene alcohol linalool, the sesquiterpenes β-caryophyllene and germacrene D, the sesquiterpenoids calamenene and cadalene, the benzenoids benzyl cyanide and methyl-salicylate. Our results clearly showed, for the first time, that apple trees strongly changed their volatile emission profile as a result of a specific, herbivore-induced response to P. heparana damage.

Keywords:*chemical ecology, plant-insect interactions, apple volatiles, herbivore-induced plant volatiles*

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O304TH11

Ecology

O304

Use of immunomarking to determine foraging patterns of *Osmia cornifrons* (Radoszkowski) in a cherry orchard

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Foraging patterns of bee species should be understood for proper placement of nest sites and to ensure adequate pollination. Field and laboratory studies were conducted to quantify the foraging patterns of Osmia cornifrons (Radoszkowski) (Megachilidae, Hymenoptera) with an immunomarking method in a commercial cherry orchard in Pennsylvania. Individuals of O. cornifrons were marked with egg-white protein powder and released from a dispenser nest placed at the center of the study orchard at early stage of bloom. After 30 h of foraging, samples were collected from randomly selected sampling cherry trees located at different distances (up to 55 m) in all directions from the O. cornifrons dispenser nest. Samples were analyzed for the presence of immunomarker protein with enzyme-linked immunosorbent assay. Foraging patterns were determined by calculating the distance and direction of marked flowers from the nest. The proportion of protein marked samples for each tree was compared with the tree's distance and direction from the nest and fruit density per limb cross-sectional area (cm2). At harvest, fruit density in the test orchard was compared with fruit density in a control orchard that did not have O. cornifrons. A high percentage of sampled flowers were protein-marked up to 40 m radius. Percentage of marked flowers dropped markedly between 41 and 55 m from the nest. Fruit density per limb cross-sectional area was significantly higher in the orchard with O. cornifrons compared with the control (P = 0.000), indicating valuable pollination services of O. cornifrons in cherry orchards.

Keywords:*immunomarking, dispersal, Osmia cornifrons, foraging, cherry orchard, pollination*

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O304TH12

Ecology

O304

Exotic omnivore dung is more attractive to native North American dung beetles than North American herbivore dung

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Although the preference of dung beetles (Coleoptera: Scarabaeidae) for specific types and conditions of dung has been given substantial attention, little has been done to investigate the potential effects of exotic mammal introduction for game farms or rewilding projects. We used pitfall traps baited with various native and exotic herbivore, carnivore, and omnivore dung to evaluate dung beetle preference in the Great Plains of North America. Additionally, we analyzed of the nutrient quality of each dung type. In total, 9,089 dung beetles from 15 species were captured in 2 years of sampling. We found significant differences ($P<0.05$) in mean dung beetle capture among omnivore, herbivore, and carnivore dung, as well as differences in individual species preference for dung type. Omnivore dung was the most attractive with chimpanzee and human dung having the highest mean capture (291.1 + 27.6 and 287.5 + 28.5 respectively). Carrion also was highly attractive with a mean of 231.9 + 20.6 beetles per trap (N=8). Our results suggest definitive local preference of carrion in Phanaeus vindex Macleay and Onthophagus hecate (Panzer), while the congener, O. pennsylvanicus (Harold), was rarely captured in carrion and highly preferred omnivore dung. Preference for a specific bait type does not appear to be correlated with dung quality, mammalian diet, or origin of mammal. Results suggest niche segregation by dung type among dung beetle species.

Keywords:*Scarabaeidae, grazing, range*

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O304TH13

Ecology

O304

Attractiveness of fermenting baits to tortricid moths in fields in Korea and New Zealand

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The attractiveness of different rice wines and fruit-based fermenting baits to various moth species was assessed in Korea and New Zealand in 2010 and 2011. In Korean apple orchards, the major lepidopteran pests such as Adoxophyes para orana, Choristoneura longicellana and Grapholita molesta were attracted to the fermenting baits. Adoxophyes para orana showed the highest trap catches with the fermenting baits in the Korean apple orchards, and four other leafroller species were also captured in the fermenting baited traps. Among the different fermenting baits tested in Korean apple orchards, 'Ga-pyung-jat' rice wine- and pineapple-based fermenting baits showed higher attractiveness to A. para orana and other leafrollers than fourteen other rice wine-based or six other fruit-based fermenting baits. In an apple orchard and a pine forest in New Zealand, the attractiveness of rice wine-, plum- and peach-based fermenting baits to Epiphyas postvittana and other moths was higher than golden syrup- or molasses- based fermenting baits. The attractiveness of the fermenting baits to Epiphyas postvittana increased when added with sucrose. The binary mixture of rice wine- and peach- or plum-based fermenting baits were more attractive to the leafrollers than the individual fermenting baits. The attractiveness of the rice wine- and fruit-based fermenting baits to the leafrollers forms the base of our ongoing efforts to develop generic attractants for leafrollers in orchards.

Keywords:*fermenting baits, leafroller, Epiphyas postvittana, Adoxophyes para orana*

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O304TH14

Ecology

O304

Would female oviposition preference affect larval anti-predator defenses? A case study using a polyphagous aquatic lepidopteran species

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In a polyphagous herbivorous insect species, host determination happens in both adult and larval stages. For those species whose neonates are considered less mobile, the ability of adult female to accurately locate and choose oviposition site is considered to affect the initial survival and development of newly hatched larvae. Here we report a case based on a polyphagous aquatic moth, Parapoynx crisonalis, whose caterpillars build portable cases with host plant leaves in order to reduce predation risk. It is expected that females lay more eggs on the plant which is suitable for larval development and case making instead of investing her eggs by chance. We firstly analyzed the growing types of the plants utilized by P. crisonalis by literature review and field analysis, and carried out a choice experiment to test whether adult female oviposition is biased on different host plants. Meanwhile, survival rate, development time of each instar, pupa mass, and average shelter building time of 10 larvae on each host species were recorded to evaluate whether adult's host plant choice corresponds with larval feeding and case-making performances. Our results suggest that while P. crisonalis were recorded feeding on a variety of aquatic plants, they were actually confined to only some floating plants probably due to its stereotypical building behavior. In addition, differences in thickness and shapes of leaves would affect the time and energy cost in shelter building and may be a main factor for host determination.

Keywords:*host selection, anti-predation, polyphagy, herbivore, Lepidoptera*

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O304TH15

Ecology

O304

Characterization of resistant factors in faba bean (*Vicia faba* L.) cultivars against cowpea aphid (*Aphis craccivora* Kosch.) using Electrical Penetration Graph (DC-EPG)

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Potential resistance of five faba bean, Vicia faba L., cultivars against cowpea aphid, Aphis craccivora Kosch. (Hemiptera:Aphidae) was characterized through colony-development study, biological study, and feeding behaviour study using Electrical Penetration Graph (DC-EPG). The last two experiment were conducted by distinguishing whole plant (WP) and detached leaf (DL) assays. Colony-development study ranked the cultivars from the highest resistance to susceptible level into Gazira 2>Commercial 'Misr'>Giza 3 improved>Goff 1>Misr 1. Total progeny number for most of cultivars except Giza 3 improved, showed significantly different between whole plant and detached leaf as shown in biological study. Feeding behaviour study revealed that the different resistant levels among five faba bean cultivars were not due to phloem tissue factors or leaf surface factor, as confirmed by the scanning electron microscope (SEM). The resistance factor especially in whole plant Gazira 2, rather because of the longer duration of stylet derailment (waveform F), however, it was not retained in detached leaf. Principle component analysis for some selected feeding parameters showed the significant difference in aphid performance between whole plant and detached leaf. The combination of colony-development and feeding behaviour studies using DC-EPG shown to be an effective way to locate the resistant factor in faba bean cultivars against cowpea aphid.

Keywords:*Faba bean, cowpea aphid, biological study, colony-development study, DC-EPG, feeding behaviour*

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O304TH16

Ecology

O304

Functional significance of fruit fly (Bactrocera: Tephritidae) male attractants and underlying genetic mechanisms

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Male attractants are widely used in the monitoring, control and eradication of Bactrocera fruit flies. Despite their importance in pest management, the functional role of attractants is known for only a few, predominantly methyl eugenol (ME) responsive, species. Most studies for ME flies suggest a role of the attractants in male courtship behaviour, with females showing preference for males that have ingested the attractants. In contrast to the better studied ME responsive species, we have studied the functional effects of cuelure (4-[4-acetoxyphenyl]-2-butanone) and zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) on B. tryoni lure-fed males and females that have mated with lure-fed males. We recorded male mating success, remating success, remating behaviour and longevity, and female mating refractoriness, fecundity, egg viability and longevity. Mating success by lure-fed males suggests preferential selection of lure-fed males by virgin females, but the effect was short-term. Further, preferential mating success was not evident when using non-virgin females. When given opportunity for daily mating, a relatively high proportion of lure-fed males remated. Females remated less after mating with lure-fed males and had higher fecundity, but there was no apparent effect on egg viability. Both lure-fed males and females mated with lure-fed males, showed increased mortality levels. We speculate the attractants are modifying sperm quality and seminal proteins that induce sexual and physiological changes in the females. We are also undertaking transcriptome analysis of lure-fed and unfed flies to correlate genetic changes with observed behavioural and physiological changes.

Keywords:*Male attractants, mate choice, sexual selection, Bactrocera tryoni, mating success, remating*

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O305TH01

Ecology

O305

Reversal in ecological host race formation in western populations of *Rhagoletis pomonella*

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A major goal of biological research is to understand how populations diverge to become separate species. The apple maggot fly, Rhagoletis pomonella (Diptera: Tephritidae), has made several important contributions to our current understanding of ecological speciation and host race formation due to its rapid host shift in the eastern United States from historically infesting native hawthorns (Crataegus mollis) to introduced apples (Malus pumila) in the last 150 years resulting in apple and hawthorn host races. Previous studies have shown that host races differ in phenology (adult eclosion time) and host odor discrimination of adult flies and exhibit large areas of genetic divergence within the genome. Recent introduction of apple flies to the western United States has initiated the development of an interesting twist to the natural history of R. pomonella when it made a reversal in host shift back from infesting apples to infesting two separate western hawthorn species (C. douglasii and C. monogyna) where there were previously no known apple maggot infestations. We conducted field experiments that provided evidence for differences in host odor discrimination between western flies infesting the three different western hosts. In addition, I have found evidence for population structure between flies infesting the three different hosts. These results provide evidence for recent genetic divergence between recently formed host associated populations., which can be a prelude to the formation of host races and is a process that has been argued to have produced the entire R. pomonella sibling species complex of five or more taxa.

Keywords:*Rhagoletis pomonella, speciation, host race formation, genetics*

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O305TH02

Ecology

O305

Genetic diversity and population structure of *Bactrocera correcta*, from China, Thailand, Myanmar and Laos based on mtDNA COI

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The guava fruit fly, Bactrocera (Bactrocera) correcta (Bezzi) (Diptera: Tephritidae) is one of the most serious insect pests of fruits and vegetables in Tropical and Subtropical regions, and regarded as quarantine pests in many countries. At present, this fly is mainly distributed in Southeast Asia. In this study, we analyzed genetic diversity and population genetic structure of the 17 populations of B. correcta collected from four countries, including China, Thailand, Myanmar and Laos using a 615-bp-long mtDNA COI sequence. Our analysis suggest that populations from Thailand showed the highest levels of genetic diversity, especially the Chiang Mai population possessed the highest levels of both haplotype diversity and nucleotide diversity, and shared an ancestral haplotype, H16. Mantel test showed that it had no isolated-by-distance effect. Fst values of all 17 populations ranged from -0.083 to 0.457, and 60.29% of it differed non-significantly, showing notable gene flow among these populations, especially between the Chiang Mai (Thailand) population and the Mengla, Kunming and Jinghong populations from Yunnan, China. This study suggests that the B. correcta populations from Thailand-Chiang Mai had a close relationship to ancestral populations. High levels of genetic diversity in Mengla indicate that this region is highly possible to be one of the entry points of this fly to Yunnan.

Keywords:*Bactrocera correcta, mtDNA COI, population structure, genetic diversity, invasion, Tephritid*

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O305TH03

Ecology

O305

Variation among ancestral populations of a global superpest: The case of the Colorado potato beetle

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Invasive species species are successful examples of rapid evolution. Approximately 40% of the major agricultural insect pests in the US are introduced insect pests that have host-shifted onto crop plants, and collectively they cause major economic losses to each year. Host shifts onto crop plants can be transformative events for insect pests, resulting in rapid evolution in response to novel selective pressures. One largely unresolved factors is the determination of whether the ability to utilize novel hosts evolves during colonization of new environments or selection upon ancestral variation. The Colorado potato beetle (CPB), Leptinotarsa decemlineata, is the most persistent and damaging potato plant defoliator within the USA, Europe, and Asia. L. decemlineata first encountered the cultivated potato, when it host?shifted onto the potato in the central US in the mid?1800's. Given its success in exploiting potato agroecosystems globally, it is striking that L. decemlineata has not established on cultivated Solanaceous plants in Mexico, the likely region of origin for the beetle. We examined if there could be geographic variation in host use among ancestral beetle populations. We have found that larvae from different Mexican populations may vary in their ability to use potato as a host. Also, adult females also vary in their ability to utilize potato as a host. Therefore, significant phenotypic variation exists among ancestral Mexican populations in terms of their ability to utilize potato as a host plant.

Keywords:*invasive species, ancestral populations, insect pests, agriculture, Leptinotarsa decemlineata,*

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O305TH04

Ecology

O305

Expression profiles of esterase genes in environmental adaptation of the brown planthopper, *Nilaparvata lugens* Stal

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To explore the possible functions and mechanisms of esterase genes in the environmental adaption of the brown planthopper, Nilaparvata lugens Stal, expression profiles of esterase genes in different populations treated with rice varieties, nitrogen applications and temperatures, respectively, were determined by quantitative PCR (qPCR). Twenty-four esterase ESTs which were previously sequenced from N. lugens cDNA library and three esterase genes available in GenBank, i.e. Nl-EST1(AF302777), AChE (AJ852420) and JH esterase (EU380769), were used to design qPCR primers, while β-actin served as the internal control gene. Effects of the rice variety including TN1 (a insect-susceptible indica variety), IR64 (a local indica variety in tropical areas), Xiushui 11 (a local japonica variety in China), and Liangyou 363 (a local hybrid variety in China), the nitrogen content in rice plants with low and high levels, and the temperature of 21oC, 25oC and 29oC on the expression pattern of esterase genes in adults of N. lugens populations collected from Zhejiang and Guangxi, China and Phitsanulok, Thailand, respectively, were highly significant. Generally, these esterase genes could be divided into 3 groups, according to their temporal expression patterns in response to environmental changes. The results confirm that esterase genes are involved in the environmental adaptation of N. lugens and have different function on the adaptation of N. lugens to environmental conditions. Cluster analysis showed a close relationship between Phitsanulok population and Zhejiang population.

Keywords:*Nilaparvata lugens, esterase gene, expression profile, rice variety, nitrogen content, temperature, quantitative PCR, environmental adaption*

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O305TH05

Ecology

O305

Effects of a plant-accumulated pollutant on the gustatory behaviors, survival and pollination ecology of the honey bee (*Apis mellifera* L.)

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Selenium (Se) has contaminated soils and plants in the western USA and other areas worldwide where pollination can be critical to the functioning of both agricultural and natural ecosystems, yet we know very little about how pollutants can impact insect pollinators. The overall goal of this study was to investigate both the sublethal and lethal effects of a plant-accumulated pollutant on a common insect pollinator. Using a plant species known to accumulate Se in the pollen and nectar, we conducted a two-year field study examining the pollination ecology of a common, weedy plant (radish, Raphanus sativus L.) and its pollinator, the honey bee (Apis mellifera L.). Se-treated plants remained attractive to honey bees and were not pollen limited. In laboratory studies, bees were fed four forms of Se (selenate, selenite, selenomethionine and selenocysteine) dissolved in sucrose solution and sucrose response threshold was quantified using the proboscis extension reflex. Inorganic forms of Se had sublethal consequences on the bee's gustatory behaviors. In toxicity bioassays, honey bee larvae were fed the same four forms of Se in artificial diet and survival was quantified. Inorganic forms of Se were most toxic to foragers, whereas organic forms were toxic at higher concentrations. Given that pollinators such as the honey bee cannot avoid Se compounds in the plant tissues they are foraging on, they will suffer similar adverse effects as seen in other insect guilds.

Keywords:*Apis mellifera, pollinators, response thresholds, proboscis extension reflex*

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O305TH06

Ecology

O305

Arsenic accumulation, sequestration, and biotransformation in aquatic Diptera

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While the affects of arsenic (As) on mammals have been widely documented, relatively little attention has been allotted to how insects are affected by and respond to As exposure. The aquatic insect Chironomus riparius (Diptera: Chironomidae) has been shown to be moderately tolerant to chronic As exposure at environmentally relevant concentrations, but the means by which it is able to withstand exposures has not been examined. As a first step in assessing detoxification mechanisms, larvae of C. riparius were reared in water containing 0 or 1000 µg As (V)/l. Early and late instar larvae, as well as pupae, adults, and egg masses were analyzed using micro-XAS to map the presence and distribution of As in the samples. Points within each sample were further analyzed to determine As speciation. Whole body imaging revealed that the majority of As appears in the midgut of early and late instar larvae, with As(V) being the major constituent. As(III) and As(III)-glutathione, an organoarsenical, were also present, indicating that the ability of C. riparius larvae to detoxify As is mediated by glutathione. Adults contained only As(V) and As(III)-glutathione, indicating the probable excretion of As in the pupal exuvia or as a meconium. Results from this study provide valuable information regarding detoxification pathways of As in C. riparius.

Keywords:*arsenate, arsenite, Chironomus riparius, micro-XAS, organoarsenical*

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O305TH07

Ecology

O305

Significance of termite mounds in heavy metal exposure.

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Many termite species construct large epigeous mounds from the subsoil. Termite mounds have been used in gold exploration as the termites have the ability to transport gold from the subsoil into their mounds. The dome-shaped mounds of Trinervitermes are a prominent feature of the savanna regions of southern Africa. In this study we tested whether during the course of building mounds T. trinervoides were transporting the heavy metal pollutants found in the soil, at two sites near unsealed tailings dams, to their mounds at the surface. Tailings dams, used to store waste generated by gold mining, are a source of heavy metal contamination in soil substrates. A profile of the heavy metal content of two medium sized mounds (30 to 40 cm in height) and the surrounding soil to a depth of 120cm was obtained. The metal content within the different termite castes was also measured. The average heavy metal content of the surface soil layer (0 to 5cm) did not differ significantly from that measured in the mounds. Thus the termite mounds are not bioaccumulating the pollutants. The mounds are built from soil collected from the surface soil layer and not from the subsoil. Thus the heavy metal contamination of the mounds was the same as the surface soil layer. The workers and soldiers appear to be accumulating copper (Cu) and zinc (Zn), however the alates, an important food source for many animals species, had very small amounts of all the metals.

Keywords:*termite mound, pollution, bioaccumulation*

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O305TH08

Ecology

O305

Transgenerational effects of heavy metal exposure on immune defense of the blow fly *Protophormia terraenovae*

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Recently environmental conditions during early parental development have been found to have transgenerational effects on immunity and other condition-dependent traits. However, potential transgenerational effects of heavy metal pollution have not previously been studied. Here we show that direct exposure to heavy metal (copper) upregulates the immune system of the blow fly, Protophormia terraenovae, reared in copper contaminated food. In the second experiment, to test transgenerational effects of heavy metal, the parental generation of the P. terraenovae was reared in food supplemented with copper, and the immunocompetence of their offspring, reared in uncontaminated food, was measured. We found that exposure to copper during early parental development stages had the same effect on the immunity of the next generation that was not exposed to heavy metal. Thus, our study suggests that heavy metal pollution has positive feedback effect through generations which multiplies the harmful effects of heavy metal pollution in following generations. Furthermore, our study indicates that changes in immunity will remain in populations even if the environmental conditions have returned to normal and the heavy metal load is no longer apparent. The results of our study suggest that the effect of an environmental toxin in populations might be more complicated than previously thought. This is something which should be considered when evaluating the effects of the pollutants on the viability of animal populations.

Keywords:*copper, encapsulation response, innate immunity, heavy metal, early development, parental effect*

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O305TH09

Ecology

O305

An invasive fruit fly benefits from a spatial and behavioural refuge against its introduced egg parasitoid

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The dynamics of host-parasitoid populations in nature are often strongly influenced by the density dependence in the interaction. Time limited parasitoids are expected to optimize their time allocation across patches often resulting in incomplete host exploitation. Additionally, hosts may create refuges through their spatial distribution, e.g. habitat choice or distribution patterns, or through behavioral and physiological defense mechanisms. We investigated the functional response of Fopius arisanus, an egg-prepupal parasitoid of tephritid flies, on egg masses of Bactrocera invadens at different host densities. The parasitism rate of F. arisanus was inversely density-dependent and followed a Type II functional response. According to Holling's theory the shape of the response results from the time a parasitoid spends handling hosts. Yet, the analysis of our data with Rogers' random-parasite equation revealed that the handling time had a minor - if any - impact on the number of parasitized hosts. Bactrocera invadens lays aggregated egg masses into the fruit pulp such that at higher clutch sizes an increasing number of eggs might be inaccessible for parasitoids. To consider the resulting spatial refuge an exponential refuge term was incorporated into the random-parasite equation. The modified model explained the observed data better than the original model demonstrating that B. invadens benefits from a spatial refuge at higher clutch sizes. As a consequence, the host refuge leads to an inversely density-dependent Type II functional response.

Keywords:*Biological control agent, functional response, host refuge, population dynamics, Bactrocera invadens, Fopius arisanus, egg parasitoid*

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O305TH10

Ecology

O305

Effects of leaf shape and host larva on oviposition preference of a tachinid fly, Sturmia bella, between two butterfly host plants

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A parasitoid fly, Sturmia bella (Diptera: Tachinidae), parasitizes more than 20 species of lepidopterans. In this study, oviposition preference and learning ability of the female were examined using Marsdenia tomentosa (Asclepiadaceae, heart-shaped leaf, a food plant of Parantica sita) and Oryza sativa (Poaceae, needle-shaped leaf, a food plant of Parnara guttata) in the laboratory. In the first experiment, we released a naive female fly in a cage with two leaves of both M. tomentosa and O. sativa without any host larvae, and recorded the number of eggs laid on leaves of each plant on the first day (Day 1). On Day 2, we presented M. tomentosa with a P. sita larva and O. sativa without any host larvae in the same way. On Day 3, we presented two plants without any host larvae again. As a result, females laid 90, 92 and 65% of total eggs on O. sativa, M. tomentosa and M. tomentosa on Days 1, 2 and 3, respectively. In the second experiment, we presented two types of paper leaf models, needle-shaped and heart-shaped ones, to naive and conditioned females which experienced a P. sita larva on Day 2 of the first experiment. Naive females laid more eggs (86%) on needle-shaped models than on heart-shaped ones, while conditioned females laid more eggs (86%) on heart-shaped models than on needle-shaped ones. The results show that this species has an ability to learn the leaf shape in connection with existence of the host larva.

Keywords:*Parasitism, learning, parantica sita*

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O305TH11

Ecology

O305

Predation on a parasitic fly: Cues arising from the host-parasite interaction increase the predation risk

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The deer ked (Lipoptena cervi L., Hippoboscidae) is an obligate ectoparasitic fly on cervids. This ectoparasite has expanded its distribution rapidly in Northern Europe during the previous decades. However, the regulating biotic factors such as predation remain unknown for this species. The host-independent pupal stage of the deer ked lasts for several months. Blackish pupae are visible against snow on the bedding sites of hosts, and are thus likely exposed to predators. To evaluate the role of predation on the population dynamics of the deer ked, we monitored pupal predation on experimental bedding sites in three geographical areas in Finland during winter. We explored: (1) possible predators; (2) magnitude of predation; and (3) whether predation risk is affected by cues arising from the host - deer ked interaction, i.e. the snow discoloration. We demonstrate that pupae are predated by a number of tit species. Reddish brown snow discoloration on host bedding sites (indicating heavy infestation of the host) serves as an exploitable cue for avian predators, thus increasing the risk of pupal predation. The ability of tits to use this cue seems to be dependent on the deer ked prevalence on the moose (Alces alces) population and the period of invasion history, which suggests that predation may be a learned behavioural response. Avian predation may thus potentially affect population dynamics of the deer ked locally.

Keywords:*ectoparasite , invasive species, predation, the louse flies, the moose*

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O305TH12

Ecology

O305

Parasitic wasp learning promotes coexistence with hosts' generation cycles in host-parasitoid system: Neural model analysis

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We have reported the effect of predator learning on coexistence of prey populations using two-host?one-parasitoid experimental systems with two bruchid beetles, Callosobruchus chinensis and C. maculatus as the hosts, and the pteromalid wasp, Anisopteromalus calandrae as their shared parasitoid (Ishii and Shimada, PNAS 2012). When the parasitoid was absent, C.chinensis was competitively excluded in approximately 20 weeks. Introduction of parasitoid greatly enhanced the coexistence time, to a maximum of 118 weeks, where C. maculates and C. chinensis showed periodic anti-phase oscillations. Behavioral experiments confirmed the frequency-dependent predation of A. calandrae, where females of A. calandrae learned the host-related olfactory cues during oviposition and increased their preference for the common host species. We simulated the learning process based on operant conditioning of the parasitic wasp that has a success-experience-reward neural system with neurotransmitters such as octopamine and/or dopamine. The individuals receive information such as which host species to and where to search from the environment. It has a neural system inside and we formulated as cycles based on cognition and behavioral action, consequences from the actions (success, reward or failure), and learning resulting in long-term memory storage. Using the model, we analyzed associative learning with conditioning stimuli (olfactory cue of each bruchid host), frequency-dependent predation of the wasp and the population dynamics with periodic anti-phase oscillations of the two hosts. In addition, we expanded the model for analyzing evolutionary dynamics using Genetic Algorithm for a three-species system with a native, an alien hosts and the wasp.

Keywords:*learning, population dynamics, three-species system, generation cycles, neural model*

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O305TH13

Ecology

O305

Endosymbiotic diversity in *Otiorhynchus* spp. (Coleoptera: Curculionidae) - a multitag 454 pyrosequencing approach

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Worldwide, several members of the weevil genus Otiorhynchus (Coleoptera: Curculionidae) are devastating pests in many horticultural crops. Notching of adult beetles on leaves and flowers as well as larval feeding on roots of their respective host plants may cause serious economic damage. Both nocturnal adult weevils and soil-inhibiting larvae are difficult to control with chemical insecticides or biological control agents. A lot of insects are associated with various endosymbiotic microorganisms and the understanding of their interactions may be used for the development of novel pest management strategies. However, before studying these interactions, a comprehensive picture of the microbial diversity and a detailed phylogeny of selected bacteria present inside the respective insect is required. Here, we used a multitag 454 pyrosequencing approach to assess the bacterial endosymbiont diversity in four different Otiorhynchus species. By sequencing in total ~48,000 PCR amplicons, 49 different operational taxonomic units (OTUs) were identified in the four studied weevil species. In a second step, for a more detailed taxonomic affiliation, specific gene regions of selected OTUs were investigated and a new clade of Rickettsia as well as "Candidatus Nardonella" endosymbionts in Otiorhynchus spp. were identified. As many endosymbionts are involved in manipulation of host reproduction or nutritional relationships, the present results are an important first step in exploring the interactions of bacterial endosymbionts and Otiorhynchus weevils and may be a basis for the development of novel control strategies.

Keywords:*454 pyrosequencing, endosymbionts, weevils*

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O305TH14

Ecology

O305

Adaptive evolution of proviral genome segments from braconid wasps obligatory symbionts

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Bracoviruses are symbiotic viruses associated with braconid wasps, which develop within lepidopteran larvae (endoparasitoid). Bracovirus particles are injected into the host with the eggs. The wasps use them to transfer and express virulence genes inside the host. The genome packaged in the particles comprises multiple circular dsDNA molecules, which are produced exclusively in the wasp ovaries from proviral sequences present in the wasp chromosomes. Bracovirus-associated wasps form a monophyletic group and bracoviruses have been chromosomally transmitted from the ancestor of the group. The comparison of bracoviruses proviral genomes gives insights into host/parasite evolutionary dynamics. We have sequenced the proviral segments of Cotesia congregata bracovirus (CcBV) and we have compared them with data obtained previously using two Glyptapanteles species separated from the Cotesia lineage ?17 million years ago. The analyses highlight the stability of the integration sites in the wasp genome identified from conserved hymenopteran genes in the flanking regions of proviral sequences, which contrast sharply with the dynamics of proviral sequences that have undergone large duplications encompassing several segments. To assess how these large genomic changes affect the coding capacity of the bracovirus genome interacting with the host, we measured the non-synonymous versus synonymous substitutions ratio in paired orthologs from four Cotesia bracoviruses (CcBV, CsBV from Kitale, CsBV from Monbassa and C. vestalis BV). This gives new information on the evolutionary arms race occurring between the wasp and its symbiont on one side and the lepidopteran host on the other side.

Keywords:*viral symbiont, polydnavirus, proviral segments, duplications, evolutionary arm race*

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O305TH15

Ecology

O305

Cytonuclear interactions and coadaption in a *Trichogramma-Wolbachia* symbiosis

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The interface between nuclear and cytoplasmic genomes represents the evolutionary arena for some of the most significant biological innovations in the history of life. The cytoplasmic symbiont Wolbachia is perhaps the most widespread bacteria bacterium in the animal kingdom with upwards of 20% of all insects infected. The ability of cytoplasmic Wolbachia bacterial symbionts to influence their nuclear hosts is clear, but the extent to which they coevolve with their host is not clear. We investigated this question using Trichogramma kaykai, a parasitic wasp in which Wolbachia induce parthenogenesis by converting haploid male embryos into diploid homozygous females. We predicted that host-symbiont combinations coevolved in the laboratory for 100 generations would have a higher fitness than novel combinations. By curing infected isofemale cultures, then re-infecting them with Wolbachia from separate cultures, we were able to compare 4 host genotypes and 4 Wolbachia "types" in a factorial design. Our results showed significant host and interaction effects for our 3 fitness variables (total fecundity, offspring sex ratio, and pupal survival) and significant Wolbachia effects on offspring sex ratio. The long-term host-symbiont combinations resulted in 9% increased higher fecundity and 2% higher pupal survival consistent with adaptive coevolution. Looking only at the non-adapted combinations, our results showed significant host effects for all 3 fitness variables (total fecundity, offspring sex ratio, and pupal survival), a significant Wolbachia effect on offspring sex ratio, and significant interaction effects on offspring sex ratio, and pupal survival.

Keywords:*Wolbachia*

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O305TH16

Ecology

O305

The nest of leafcutter bee *Megachile unbripennis* Smith (Megachilidae) in Bangka Island, Indonesia

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Understanding of the biological role is needed for managing its ecological role and its benefit for human being and ecosystem.Nest construction, building nest duration, plant species, cutting behavior, leaf cutting duration, cutting direction, leaf position and age preference, cut leaf form and area, and cutting time preference from rose leafcutter bee, Megachile unbripennis Smith (Megachilidae) in Sungailiat (Bangka Island) and Yogya (Java Island) were studied. The nest shape of M. unbripenniswas tube, 39 mm length, 8 mm diameter at rear,11 mm diameter in the middle, and 9 mm diameter at the opening. It consisted of 22 - 32 leaf cuts in various sizes between 36 - 223 mm2 with the average of 147 mm2 and was located in the abandoned banana skipper caterpillar's nest, Erionata thrax l. (Hesperiidae). The leaf cuts that constructed the nest werein various sizes depended on their position on the nest. The nest building was relied on season, weather, and temperature.The most suitable season is early until mid dry season or around April - May, bright days, the average rain falls 273 - 181 mm, the average temperature 27oC, and the average of relative humidity is 80.6%. Beside rose leaf (Rosa sp.)(Rosaceae), 13 other plant speciesof 11 families of herbs, shrubs, and trees werecut by the bee. The more frequently cut were on Syzygium polyanthum (Myrtaceae), Bougainvillea spectabilis (Nictaginaceae), and Pterocarpus indicus (Fabaceae).

Keywords:*leafcutter bee, Megachile unbripennis, nest shape, Bangka Island*

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O305TH17

Ecology

O305

Comparison of stream macroinvertebrate communities from two Philippine Islands: A biogeographic study

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Geographical patterns in the distribution of macroinvertebrates were investigated in river systems located in the Mindanao and Leyte Islands. During the late Pleistocene, these two islands were connected and together with Bohol, Samar, DInagat and many other smaller islands form part of the Greater Mindanao Island. Species distribution of mammals, reptiles, amphibians, and freshwater fish provide a strong evidence for the connection of land bridges during the Pleistocene. However, studies testing this connection of Philippine islands using freshwater macroinvertebrates are rare. Consequently, the aim of this study was to investigate if there were differences in the macroinvertebrates community composition between the two islands. Nonparametric multidimensional scaling (nMDS) ordination of samples (macroinvertebrate abundance/site) was performed using Bray?Curtis similarity coefficient. Visual distribution assessment suggested two island biogeographic groups (albeit weak; stress value = 0.19), with a small region of overlap indicating that several taxa are being shared. Nonetheless, differences in macroinvertebrate assemblages were found between islands (ANOSIM R = 0.266, P = 0.002). Of the 75 taxa identified, only 28 (37%) were found in both islands. Further studies integrating molecular phylogenetics and DNA barcodes of stream macroinvertebrates could improve descriptions of community structure and thus provide greater discriminatory ability regarding biogeographic distribution of macroinvertebrates between the two Philippine islands.

Keywords:*Biogeography, Greater Mindanao island, macroinvertebrates*

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O305TH18

Ecology

O305

Invertebrates mediate the responses of decomposer fungi to elevated temperature

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Elevated temperature has potential to influence the biological mechanisms regulating ecosystem-atmosphere carbon exchange. The relationship between warming and heterotrophic microbial respiration remains poorly understood, mainly due to uncertainty regarding the differential sensitivity of microbial groups to temperature and the complexity of interactions with other biota. Grazing soil invertebrates influence the biomass and composition of microbial communities. Cord-forming basidiomycete fungi dominate primary decomposers in temperate woodlands. Warming is expected to stimulate mycelial growth and decomposition, but could also increase the abundance of mycophagous collembola. Increased grazing pressure at elevated temperature is likely to mediate the fungal response to warming. We investigated the impact of elevated temperature on fungal foraging and decomposition of beech (Fagus sylvatica) wood in soil microcosms to which mycophagous collembola were added in factorial combinations with five basidiomycete fungi. Species-specific impacts on mycelial development and function resulted from differential sensitivity of fungi to warming and grazing. Temperature impacts on collembola abundance were resource-specific, causing increased grazing pressure by both species, but on different fungi. When a fungus was palatable to a particular collembola species, increased grazing pressure counteracted warming-induced stimulation of mycelial growth, with implications for colonisation rates of new resources. High grazing pressure did not prevent increased fungal-mediated decomposition of colonised wood, as fungi utilised more resource-derived energy to maintain explorative growth. Impacts of elevated temperature on decomposition are likely to depend on local composition of the fungal and invertebrate decomposer community.

Keywords:*climate change, fungus-invertebrate interactions, decomposition*

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O305TH19

Ecology

O305

The trial and performance of an international joint study between Korea and Russia for the restoration of Callipogon Relictus

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Center for the Study of Insect Ecology (CSIE, Yeongwol, Korea) entered into a long-term, amicable and mutual agreement for a joint study on Callipogon relictus with FEB RAS (Far East Branch of Russia Academy of Science) in 2006. Callipogon relictus is distributed to the north around North Korea including boundaries of China, boundaries of Russia, especially around Ussuriysk, and to the south around some areas of Gyeonggi-do Province and Gangwon-do province near boundaries between South and North Korea as the southern limit. Callipogon relictus in a large size reaching 8~10cm for the average body length is protected in Russia as it is described in the Red Book, while it is designated as Extinction Crisis Class 1 and our natural monument in Korea. While the population is relatively abundant in Russia, it is considered that it has reached the level of actual extinction in Korea. The first joint study was commenced as a study agreement was entered into between CSIE and Ussurii Reserve in 2007. Both organizations have studied the larval stage of Callipogon relictus without dormancy while breeding larvae of Callipogon relictus respectively in their laboratories. Both organizations adopted the breeding methods in a different way. Ussurii Reserve has seen the emergence of 3 imagoes in 3 years and 8 months in April 2011, as a result of breeding larvae under the conditions of temperature 20 and humidity 60% using raw timber of Ulmus in 50cm length and 60cm diameter since August 2007. On the other hand, CSIE in Korea

Keywords:*Callipogon relictus, International Joint Study*

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O305TH20

Ecology

O305

Activity of insect fauna during the night, in the palm orchards of central Iraq

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Ministry of Science & Technology, Iraq

The study was carried out at three palm orchards, each of 2.5 hectare in Al-Madain palm orchards(30 km. South Baghdad) contain the palm varieties (Prem, Osta omran, Zahdi , Berhee, Khadrawi , Khastawi ,Oueedi and Tebarzel) in 2010 Season aiming to determination of population density of Palm Borers and to know other insect occurring in Date Palm orchards. Three light traps with solar energy (Magna Traps with lamps of 320 ? 420 nm wavelength are produced by Russell IPM) were used as one trap in each orchard. The number of Fruit Stalk Borer, Oryctes elegans and Frond Borer, Phonapate frontalis adults were calculated per week in each trap aiming to determine the population density of adults in the three orchards under this study. Results of using light Traps (Magna Traps) indicated that the number of O. elegans adults catch per trap during the period from April to December 2010 were 254 , 217 and 240 adult per trap in orchards No. 1, 2 and 3 respectively , the higher population densities were 87 ,79 and 81 adult /trap per month, respectively reported in July. Also, The study indicated that the population density of Frond Borer, Phonapate frontalis catch per Trap during the period from April to December 2010 were 34, 58 and 54 adult /trap for orchards No. 1, 2 and 3 respectively, The higher population densities were 10, 21 and 15 adult/trap per month, respectively reported in June.

Keywords:*Insect, Population density, Palm,Borers, Light trap.*

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O305TH21

Ecology

O305

Comparative studies of behaviourial variations of *Apis mellifera* L. species in Nigeria

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230 words Studies were conducted for three beekeeping seasons (between 2009 and 2011) on 400 colonized hives sited in the two major vegetation zones of Nigeria. Direct counting of the number of sting babels deposited on the beekeepers kit by scout bees defending her colony was done during the period of routing inspection and harvesting of crops from selected colonies while swarming and abscondment of established colonies after the first visit was noted and recorded for each farm. Behaviourial variations of Apis mellifera species from the forest and savannah vegetation zones of Nigeria revealed that majority (above 65%) of the honeybees were highly aggressive and none of the colonies in the country was stingless. The preponderance of the deposit of the sting babels by the scout bees in the nation was highly aggressive +++> aggressive ++ > less aggressive + >stinglessness ?. Comparison of behaviourial variations of Apis mellifera colonies in Nigeria revealed that Abuja (31.3%), Adamawa (4.0%) and Osun (3.3%) States had the less aggressive species of honeybees while, both Kebbi (80.7%) and Ebonyi (79.7%) States recorded the highest percentages of sting babels deposition in the savannah and forest vegetation respectively. The percentage of abscondment and swarming

Keywords:*Apis mellifera, Colonies, Aggressiveness, Swarming, Abscondment*

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O305TH22

Ecology

O305

Circadian control of oviposition and hatching in *Epilachna vigintioctopunctata* (Coloeoptera: Coccinellidae): A laboratory study

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The ladybird beetle belongs to the family Coccinellidae of order Coleoptera. The members of the family are solely predator on aphids, mealybugs, scale-insects, whiteflies, thrips, leafhoppers, mites and other small soft bodied insect pests. It is known to prey on about 39 arthropod species. The predatory coccinellids occupy all the habitats and niches of their preys and are dispensed worldwide. Epilachninae a subfamily of Coccinellidae is exemplified by existence of phytophagous species which are pests of imperative agricultural crops and their range widens throughout India, Pakistan, China, Japan, Southeast Asia and Oceania. The members bear close semblance in size, appearance, overlapping distribution and equivalent food habits so are deemed as cost-effectively significant beetle pests. Epilachna vigintioctopunctata (Fabricius) recurrently recognized as the 28-spotted ladybeetle or the hadda beetle is a very frequent pest of nightshades and cucurbits. The nature of damage varies with the geographical regions where both the adults and the grubs are accountable for declination of the crop yield. Normally the crest is found in July and August. Both adult beetles and grubs feed on the epidermal tissues by scrapping, ensuing drying up and falling of the leaves due to which they are sometimes called leaf scrapping coccinellid beetles. The study also spotlights that food plays a momentous effect on oviposition and age of female also casts its spell on oviposition. Oviposition was found to augment as age of female amplifies, attains its crest and then ultimately declines.

Keywords:*Epilachna vigintioctopunctata, oviposition, circadian rhythms*

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O305TH23

Ecology

O305

Distributions and seasonal fluctuations of carabid species (Coleoptera: Carabidae) of hazelnut plantations in Turkey

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The family Carabidae, or ground beetles, is one of the largest families of Coleoptera. They are important natural enemies in agroecosystems. There are approximately 600,000 ha of hazelnut plantations in Turkey that usually feature continuous groundcover and minimal disturbance by machinery. Pitfall traps were established in June 2011 at 17 sites along the eastern Black Sea coast from Samsun Province to Trabzon Province. Trapping ranges over a distance of 350 km from east to west and to 450m altitude. The three traps at each site were checked every 2 weeks in spring, summer and autumn and 3-4 weeks in winter. To date, 32 species from 15 genera have been collected. Pseudoophonus rufipes was the most common and widely distributed species, following by Brachinus explodens. From the well known predatory genus Carabus, C. chevrolati chevrolati, C. wiedemanni anceps and Carabus spinolae salignus were the most common. Carabus (Procrustes) chevrolati chevrolati was the most widely distributed species. Carabus robustus triumphans and Carabus bonvouloiri bonvouloiri, an endangered species, were also collected. Numbers of species and their distributions varied considerably from site to site, including altitude, and abundance was greatest in summer and autumn. Some species continued to be active during warm winter weather.

Keywords:*Predator, carabid, population density, distribution, hazelnut, Black Sea*

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O305TH24

Ecology

O305

Effects of the host plant on the biology and life-table parameters of two-spotted spider mite

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Studies on the biology and life-table parameters of two-spotted spider mite, Tetranychus urticae Koch on three leguminous hosts, Common bean, Phaseolus vulgaris, Vetch Vicia sativa and Soybean Glycine soja revealed indicated that the duration of different developmental stages of T. urticae were 3.94, 2.69 and 2.93 days for eggs, 1.26, 2.1 and 1.96 days for larvae and 2.22, 3.38 and 2.67days for nymph, respectively. The fecundity were 23.04, 35.63 and 33.92, eggs/female, while pre-oviposition, oviposition and post-oviposition periods lasted for 2.89, 3.62 and 0.21 days on common bean, 1, 4.13 and 0.59 days on vetch 0.7, 3.80 and 0.4 days on soybean, respectively. The total developmental period from egg to adult was recorded as 7.42,7.56 and8.17 days and longevity of male were 4.71, 4.17and 3.55 days and that of female were 5.76, 4.9 and 3.94 days. Demographic parameters (Ro, rm , λ ,T and DT) of T. urticae were 8.523, 0.178, 1.194, 12.065 and 3.903 on common bean, 4.456,0.159,1.163, 9.885 and 4.587 on Vetch and 6.783, 0.209,1.233, 9.158 and 3.316 on Soybean.

Keywords:*Host plant, Biology, life-table parameters, Tetranychus urticae.*

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O306F01

Ecology

O306

Heritable variation of life-history traits in an adapted spruce budworm population

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The spruce budworm is a major pest in the boreal forest of North America; this native insect exhibits eruptive behaviour in approximately 30-year cycles. There is no explanation of what causes the populations in the forest to rise and fall; it could be due to coevolution processes. There are two components involved in adaptive evolution: natural selection acting on the phenotype, and genetic variability in phenotypic traits. The integration of these components may provide information about causes and constraints on evolution in contemporary populations. Understanding heritability in the spruce budworm could be key to answering fundamental questions about its adaptation. The purpose of this study was to estimate the heritability of life-history and metric traits in the spruce budworm. To avoid environmental variability, budworms were reared on an artificial diet and under laboratory conditions. We estimated heritability by parent-offspring regression analysis of the following traits: fecundity, egg fertility, developmental time, and pupal mass weight. Results indicate that the population maintains substantial additive variance in life-history traits like other groups of Lepidoptera, and low heritability in pupal mass weight. This suggests that natural selection on spruce budworm is probably higher during the winter period. This information is very useful in predicting heritable variation under epidemic conditions.

Keywords:*spruce budworm*

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O306F02

Ecology

O306

A test of genetic theories underlying Haldane's Rule with haplodiploid wasps

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Haldane's Rule describes a pattern early during speciation in which the hetero(hemi)gametic sex suffers stronger from hybridization than the homogametic sex. Three main genetic theories try to explain Haldane's Rule: the dominance, the faster-male and the faster-X theory. The question is whether the hetero(hemi)gametic sex is more susceptible to hybrid incompatibilities due to its sex or its hemizygosity of the sex chromosome. We show how haplodiploid organisms (haploid males, diploid females) are uniquely suited for studying the genetic basis of Haldane's Rule. We constructed sex-reversed diploid males of the haplodiploid wasp Nasonia vitripennis and compared their levels of hybrid incompatibilities with haploid males and diploid females. Inviability and sterility was low in diploid males and females compared to haploid males indicating the absence of sex-specific incompatibilities. Genomic regions associated with hybrid mortality were similar in diploid males and females, but less frequent than in haploid males. Dominance effects were found in some regions, but homozygosity of incompatible loci was mostly rescued by diploidy in hybrid males and females. Dosage effects of incompatibilities is a novel phenomenon for explaining Haldane's Rule. It may be specific for haplodiploids, but could also apply to X-linked incompatibilities in diploid species.

Keywords:*haplodiploidy, incompatibility, speciation*

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O306F03

Ecology

O306

Joining forces in defense: Production dynamics of symbiont-mediated antibiotics reveal long term antimicrobial protection for wasp offspring

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Bacteria-produced antibiotics play a pivotal role in pharmacology to treat a plethora of microbial infections. Recently, insects have also been found to benefit from these antimicrobial properties to defend the host or its nutritional resources against pathogens. In the solitary digger wasp Philanthus triangulum, a cocktail of nine different antibiotics, produced by Streptomyces symbionts, inhibits the growth of detrimental microorganisms and significantly enhances the offspring´s survival probability. Using GC-MS and RT-qPCR we investigated the dynamics of antibiotic production during beewolf development. The analyses revealed a relatively constant Streptomyces population size over time. By contrast, we found a significant change in the antibiotic amount, with a strong increase of antibiotics within the first two weeks after cocoon spinning, and a slow subsequent decrease, indicating that antibiotic production is optimized to serve as a reliable long term protection during the unpredictable developmental phase of beewolf larvae.

Keywords:*Streptomyces, beewolf, symbiosis, hymenoptera, antibiotics*

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O306F04

Ecology

O306

Predation selects for lower resting metabolic rates in *Tenebrio molitor*

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The energetic definition of fitness predicts that natural selection will maximize the residual energy available for growth and reproduction, suggesting that energy metabolism might be a target of selection. In this experimental study we investigated whether female and male yellow mealworm beetles (Tenebrio molitor) differ in their hiding behaviour, individual response latency time, and duration of immobility to treatments mimicking an approaching predatory threat. We experimentally tested whether consistently repeatable anti-predatory responses and resting metabolic rates (RMR) correlated with survival rates of individuals exposed to a nocturnal predator, the Norwegian rat (Rattus norvegicus). RMR was part of a complex syndrome involving anti-predator behavior. Individuals with lower RMR concealed themselves against predators in the layer of bran more often than individuals with higher RMR, and hiding was associated with longer periods of immobility. Our results provide direct evidence of natural selection against elevated RMR in T.molitor beetles.

Keywords:*Resting metabolic rate, Tenebrio molitor, Natural selection, Personality*

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O306F05

Ecology

O306

Temperature-dependent, behavioral, and transcriptional variability of a tritrophic interaction consisting of bean, herbivorous mite, and predator

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Different organisms compensate for, and adapt to, environmental changes in different ways, and therefore environmental changes affect animal?plant interactions. We assessed the effect of temperature on a tritrophic system consisting of the lima bean, the spider mite Tetranychus urticae, and the predatory mite Phytoseiulus persimilis. In this system the plant defends against T. urticae by emitting volatiles that attract the predatory mite P. persimilis. Over a temperature range of 20°C to 40°C the emission of volatiles by infested plants, and the attraction of P. persimilis, peaked at 30°C but the number of eggs laid by T. urticae adults, and the number of eggs consumed by P. persimilis, peaked at 35°C. This finding indicates that the temperature at which both spider mites and predatory mites performed optimally was higher than the temperature at which the volatile attractants were produced at the greatest levels. We used data from transcriptome pyrosequencing of the mites and found that P. persimilis up-regulated gene families for heat shock proteins (HSPs) and ubiquitin-associated proteins, whereas T. urticae did not. RNA interference-mediated gene suppression in P. persimilis, developed in the current study, revealed that the strongly induced PpHsp70-1 contributed to the predation of T. urticae eggs at 35°C but not at 25° C. Overall, our molecular and behavioral approaches revealed that the mode/tolerance of lima bean, T. urticae, the predatory mite P. persimilis are distinctly affected by temperature variability, thereby causing temperature-dependent, mutualisms in the tritrophic level.

Keywords:*heat shock protein (HSP), lima bean, predatory mite, spider mite, transcriptome pyrosequencing*

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O306F06

Ecology

O306

Biology of coconut black-headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae), records of alternative host plant and its natural enemies in Thailand

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Due to the devastating outbreak of the black-headed caterpillar, Opisina arenosella Walker in Southern Thailand in 2010, gathering the information of the pest insect is essential. Accordingly the insects were reared on their alternative host plant, banana leaves, to study their biology. Female adults laid yellowish creamy color eggs in mass. The newly hatched larva was orange-red and then turned to pale yellow, with dark brown head. Three brown lines ran along the lengthwise of the body. Thorax color was paler than that in head and legs. The oval shape pupae were dark brown. Head, antennae, wings and abdomen of adult were light gray. Female was larger than males. Average number of egg laid was 83.40±14.31 /female until death. Egg duration averaged 4.90±0.55 days. Larvae of O. arenosella had 10 to 13 instars with a larval period 57.67±9.25 days. The pupal period was 9.08±0.90 days. Longevity of male and female were 10.33±5.01 and 9.00±1.22 days, respectively. Mean duration life cycle was 80.45±10.46 days. Further study on alternative host plants of O. arenosella in Nakhon Pathom and Prachuap Khiri Khan provinces, Thailand from November 2010 to August 2011, disclosed a total of 11 species; 10 species of Palmae and one species of Musaceae. Additionally, five species of parasitoids (Hymenoptera: Braconidae, Chalcididae, Eupelmidae, Eurytomidae and Eulophidae) and two of insect predators (Hemiptera: Pentatomidae; Dermaptera: Chelisochidae) were collected in the outbreak areas.

Keywords:*Opisina arenosella Walker, biology, natural enemies*

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O306F07

Ecology

O306

Slow-growth-intraguild predation- status; when the diet determines your IGP status...

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The slow-growth-High-mortality hypothesis states that organisms with a slower development will suffer a higher mortality than organisms with a shorter development. Omnivorous natural enemies can exploit an array of different food sources, from animal and plant material. These food sources being of different qualities would affect the growth, development, vigor and survival of the insects. More specifically, a low-quality diet will extend the larval developmental time, and at a specific time have produced smaller individuals. Consequently, we formulated the hypothesis that, in an intraguild interaction context, the diet will determine the IGP status of the focal natural enemies. We predict that individuals fed with a high-quality diet will be more often intraguild predators than individuals fed with a low-quality diet. We worked with two north-American ladybeetle species at the larval stage. The twelve-spotted ladybeetle was fed either on a high or low-quality diet and confronted to the Asiatic ladybeetle. Results will be discussed considering priority factors and overwintering behaviors.

Keywords:*diet, SGHM, zoophytophagy, omnivory, Coccinellidae, IGP, direction, interaction*

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O306F08

Ecology

O306

Evolution of cooperation: Insights from a tiny haplodiploid insect.

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Cooperation in nature is a paradox for behavioural ecologists, who interpret most behaviour as fundamentally selfish. Species that can choose whether or not to cooperate are especially helpful in understanding how this behaviour evolved. Tiny female Acacia thrips (Dunatothrips aneurae) often cooperate to build nests from tied phyllodes of mulga (Acacia aneura). But this carries a cost: cooperating females get fewer offspring than solitary females. Evolutionary logic suggests there must be a benefit to counterbalance this cost. Cooperation in thrips is especially interesting because they are haplodiploid, and so represent a genetic parallel to similar well-studied systems in the Hymenoptera. However, very little is currently known about the basic natural history of this species. We explore the natural history and cooperative behaviour of D. aneurae in the field and lab. D. aneurae domiciles were highly patchily aggregated. The thrips showed no aggression of any kind. Invading inquiline thrips disrupted reproduction by competing for resources within domiciles. Mating among nestmates was common. Females required male presence to initiate domiciles. Cooperation was more readily induced simultaneously than by females joining nests sequentially. Larger nests contained middens of discarded waste. Cooperation was associated with (i) higher per capita survival, (ii) a narrower range of phyllode dimensions in domiciles, (iii) local crowding and/or east-facing orientation on trees, (iv) reduced hydroxyproline in phyllodes, indicating reduced drought stress. D. aneurae and its domicile-building congeners promise to become an interesting new model clade for asking questions about how and why cooperation evolved.

Keywords:*sociality, altruism, Hymenoptera, Thysanoptera, behavioural ecology*

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O306F09

Ecology

O306

Winter host selection influences fitness traits in an aphid parasitoid

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Organisms can either evade winter's unfavourable conditions by migrating or diapausing, or endure them by maintaining their activities. When it comes to foraging during winter, a period of scarce resources, there is a strong selective pressure on resource exploitation strategy. Generalist parasitoids are particularly affected by this environmental constraint, as their fitness is deeply linked to the profitability of the chosen hosts. In this study, we considered a cereal aphid-parasitoid system and investigated (i) the host-parasitoid community structure, host availability and parasitism rate in winter, (ii) the influence of host quality in terms of species and instars on the fitness of the aphid parasitoid Aphidius rhopalosiphi, and (iii) whether host switching has an impact on parasitism success for that parasitoid species. Host density was low during winter and the aphid community consisted of the species Rhopalosiphum padi and Sitobion avenae, both parasitized by A. rhopalosiphi at non-negligeable rates. A. rhopalosiphi produced offspring more often when parasitizing R. padi when compared to S. avenae, but the latter aphid species originated significantly bigger parasitoid offspring. Though aphid adults and old larvae were significantly larger than young larvae, these last ones resulted in both higher emergence rates and larger parasitoids. Host switching did not bear any detectable negative consequences on parasitoid emergence rates or offspring body size. This study provides some evidence that winter A. rhopalosiphi populations are able to take advantage of a large array of host types that vary in profitability, indicating that host electivity drops under winter's unfavourable conditions.

Keywords:*Profitability, Aphidius rhopalosiphi, life-history traits, winter, host quality*

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O306F10

Ecology

O306

Effect of mating interactions between females and males from different primary host species on host feeding and parasitism by the autoparasitoid *Encarsia sophia*

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The autoparasitoid Encarsia sophia not only parasitize whiteflies but also kill them with strong host-feeding capacity. In this study, E. sophia females and males were reared on primary hosts, Trialeurodes vaporariorum and Bemisia tabaci 'Q', and the host-feeding and parasitism of wasps on both whitefly species were determined in all different mating combinations: (i) E. sophia female reared on B. tabaci (ESF-BT) mated with E. sophia male from B. tabaci (ESM-BT), (ii) E. sophia female reared on T. vaporariorum (ESF-TV) mated with E. sophia male from T. vaporariorum (ESM-TV), (iii) ESF-BT mated with ESM-TV, (iv) ESF-TV mated with ESM-BT. ESF-TV mated with ESM-TV killed most whitefly nymphs of B. tabaci and T. vaporariorum through host feeding. The larger ESF-TV in body size mated with larger ESM-TV killed more whitefly nymphs through host feeding than those mated with smaller ESM-BT. Whether B. tabaci or T. vaporariorum were used as hosts, ESF-TV mated with ESM-TV and ESM-BT, and ESF-BT mated with ESM-TV significantly parasitized more whitefly nymphs than ESF-BT mated with ESM-TV. In general, the mating combination of female and male originated from T. vaporariorum caused largest mortality of whitefly through parasitism and host feeding on both whitefly species, but the combination of ESF-BT mated with ESM-TV killed fewest whiteflies. These results indicated that the performance of autoparasitoids on insect pests was not only dependent on females, but also affected by mating males from different primary host species.

Keywords:*Encarsia sophia, Bemisia tabaci, Trialeurodes vaporariorum, hyperparasitoid, host feeding*

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O306F11

Ecology

O306

Morphological changes correlated with horsehair worm infection in *Hierodula formosana* Giglio-Tos (Mantodea: Mantidae)

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Parasitism is a strong driving force of evolution for both hosts and parasites. This long-term coevolution usually causes unusual phenotypes in the infected hosts. Parasite-correlated alterations of host morphology have been recorded in many host-parasite associations. Since the morphology reflects the development and the selective pressure within a given environment, it is also a good index to know the strategy of both parasites and hosts. Hierodula formosana is a common mantid species in Taiwan, and their adults are always found to harbor the parasitic horsehair worms, Chordodes formosanus. In this study, we compared the external morphology of Hierodula formosana, including both sexes and infected individuals. The infected mantids were found to have smaller body sizes and the relatively shorter wings. Additionally, shorter mid legs and hind legs, and lower density of antennal sensilla were also found on the infected males. In conclusion, allometry in the mantid hosts somehow implied the decrease in locomotion ability as well as the interference in development by parasites. As the reduced number of antennal sensilla happened in our infected mantid hosts was also found in some juvenile insects like cockroaches, we also believe this probable parasite-induced juvenilization may have widely appeared in many parasitism cases to avoid adults investing too much energy in dispersal and reproduction.

Keywords:*Nematomorpha, mantids, host manipulation, allometry*

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O306F12

Ecology

O306

Ecological plasticity and genetic diversity of the mango blossom gall midge, *Procontarinia mangiferae* (Felt), in Reunion Island

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Phytophagous insects that belong to the Cecidomyiidae family are among the most closely associated with their host plants. Phytophagous insects can specialize not only on a unique host plant but on particular parts of plants as well. Mango blossom gall midge, Procontarinia mangiferae, is a mango pest that causes damage to both inflorescences and leaves. The objectives of our study were to highlight the genetic diversity and ecological abilities of a monophagous gall midge and to evaluate the determinants of its genetic structure in subtropical Reunion Island. This study, carried out on an isolated island, is based on morphological and molecular data (mitochondrial DNA and microsatellites). Using mitochondrial COI gene sequences, polymorphism at 11 microsatellite loci and an extensive sampling of 27 populations at 17 sites, we tested the genetic differentiation between populations sampled on different mango organs, different mango cultivars and at different seasons. Our results showed that a single species, P. mangiferae, was present all year round at all of the sites sampled, regardless of climatic and cultural conditions, and that it fed on both inflorescences and young leaves. Moreover, our study highlighted the ecological plasticity of this species. However, unlike previous studies on other Cecidomyiidae species, the population genetic structure did not appear to be determined by the species' ability to feed on different organs of the same host plant, but rather by the geographical distribution of populations.

Keywords:*Cecidomyiidae, Ecological genetics, pest species*

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O306F13

Ecology

O306

Are sneaky males making the best of a bad lot or reaping the rewards? Alternative reproductive tactics in the giraffe weevil (*Lasiorhynchus barbicornis*).

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Many animal species have evolved weaponry as a means to resolve conflict between conspecifics in the acquisition of mates. Males with the largest weapon or body size are generally thought to have a competitive advantage during conflict. However, in some species where there is high adult size variation, small males employ alternative reproductive tactics (ARTs) that do not rely on weaponry to gain access to females for copulation. These tactics are often in the form of sneaking by small males, versus guarding by large males. Despite these ARTs, subordinate males are usually thought to have a lower mating success than dominant males, and are simply "making the best of a bad lot". Males of the giraffe weevil (Lasiorhnychus barbicornis) (Coleoptera: Brentidae) have an elongated rostrum which is used as a weapon to compete with other males to secure females for copulation. However, males are also highly size variable such that there is a 6-fold difference between the smallest and largest equivalent-aged individuals. We found that small males, in addition to using their rostrum to fight, will also use sneaking tactics to copulate with females while larger males stand guard. To determine if body size is a determinant of mating success, and to investigate the influence of both seasonal and local density on the relative mating success of different sized individuals, we conducted an extensive series of field-based observations between 2010 and 2012. These results will be discussed in the context of sexual selection, competition, and alternative reproductive tactics.

Keywords:*mating behaviour, alternative reproductive tactics*

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O306F14

Ecology

O306

Gall formation strategies of *Idarnes* group *flavicollis* associated to *Ficus citrifolia* in Brazil

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We studied the galling process of a non-pollinating fig wasp species Idarnes group flavicollis associated to Ficus citrifolia in Brazil in both pollinated and unpollinated figs between 2010 and 2011. For pollinated treatment, simultaneous to wasp oviposition we injected a sucrose 5% solution with pollen through the fig ostiole. We conducted pollinated and unpollinated treatments in two and three F. citrifolia trees, respectively. From oviposition day we sampled 4-5 figs each two days until larva completed development. For each sample, we dissected 20 randomly-picked galls; we measured larva total body length and recorded its position inside the gall. We also destined 10-20 developing galls and seeds at four-day intervals for histological study. Female deposits its egg between ovule inner integument and nucellus. First larval stage (0.206±0.059mm, n=60) begins to feed on hypertrophied nucellus. In pollinated oviposited flowers, endosperm and plant embryo cells suffered hypertrophy which interrupted its normal development. Larva position changed in second larval stage (0.745±0.207mm, n=60) to the micropillar region. Second larval stage fed on hypertrophied cells originated from either nucellus or endosperm/embryo. Third larval stage (1.507±0.291mm, n=80) continued to grow until it occupied the whole gall cavity. We show that although this Idarnes species oviposits at a same fig developmental stage as the pollinator and emulates the location where pollinator's egg is deposited, galling strategies differed considerably from it. Successful gall formation in Idarnes group flavicollis is independent from plant fertilization. Financial support: S.J.G. FAPESP#09/10237-9. R.A.S.P. FAPESP#04/10299-4 and CNPq#302769/2008-0. S.P.T. CNPq #301960/2009-7.

Keywords:*Gall formation, non-pollinating fig wasp, larva, Ficus, Chalcidoidea*

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O306F15

Ecology

O306

Does overheating risk affect the evolution of cryptic coloration?

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Cryptic coloration has multiple functions, such as thermoregulation and /or signaling, which are potentially incompatible each other. Here we focus on rarely explored trade-offs between camouflage and thermoregulation. A polymorphic pygmy grasshopper Tetrix japonica exhibits male-limited absence of black markings, despite of their enhanced camouflage effect. The grasshopper males search for females under strong sunlight in summer. According to the thermal melanism theory, ectothermic melanistic individuals get warm and reach higher equilibrium temperatures faster than lighter ones. Therefore, the black markings of the grasshopper males are expected to incur the overheating risk. We hypothesized that the non-marked males took an overheating avoidance strategy. Choice experiments for radiation heat in the laboratory revealed that non-marked males stay longer under radiation heat than marked males did, though the body temperature of the two morphs was quite similar when they started to hide from the radiation heat. These results suggested that non-marked males got less warm and could stay longer for mate searching than marked males did. Namely, non-marked males were advantageous in hot conditions. This was strongly supported by the altitudinal and latitudinal clines, respectively at regional and geographic scales, with greater proportion of the non-marked males in lower and southern areas.

Keywords:*thermoregulation, grasshopper, cryptic coloration*

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O306F16

Ecology

O306

Plastic defensive behavior of termite workers depending on social context

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Social insect colonies are self-organized systems that flexibly respond to environmental changes such as a single organism. By altering the caste proportions, social insects change the task allocation according to colony demands. In this sense, the differentiation of termite soldiers, which are morphologically specialized to colony defense, is regulated depending upon requirements like caste proportion of colonies. However, the soldier differentiation from larvae (or workers) requires a relatively long time to be accomplished. To achieve flexible and emergent colony defense, other rapid-response mechanisms can be predicted. To test our prediction, we quantified the defensive responses of soldiers, pseudergates (workers) and reproductives of the damp-wood termite Hodotermopsis sjostedti. When the isolated castes were exposed to an intruder, soldiers exhibited the most vigorous defensive response, followed by pseudergates and then reproductives. Under paired condition, while the aggression levels of soldiers and reproductives were independent of the accompanying castes, pseudergate aggression was more plastic and depended on the other castes present. When paired with reproductives, pseudergates exhibited high levels of aggression towards enemies, while the pseudergate aggression remained low when they were paired with soldiers. The results indicate that pseudergates modulate their defensive response depending on social context, possibly contributing rapid and flexible responses required for colony defense. To elucidate the underlying physiological mechanisms, we further investigated the contributions of biogenic amines on the defensive behavior of pseudergates. The results suggest that tyraminergic/octopaminergic systems regulate the aggression levels of pseudergates, indicating the possible regulatory roles in the social defensive responses of termite colonies.

Keywords:*social insect, termite, aggression, biogenic amine, colony defense, social behavior*

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Ecology

O306

Age-related male reproductive investment and female fitness consequences in a moth, *Ostrinia scapulalis*

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Energy spent on current reproduction could reduce the potential for future reproduction since energy is a limited resource. According to the terminal investment hypothesis, costly reproductive investments should be increased toward the end of a lifespan when the probability of reproduction and survival becomes low. We investigated age-related changes in male reproductive investment toward courtship display and the spermatophore in a monandry moth, Ostrinia scapulalis. We found that older males had higher mating success than younger males under both choice and no-choice conditions. Observations of behavior in the two-choice (1- and 3-day-old male) test revealed that older (3-day) males courted more frequently than younger (1-day) males, but there was no difference in rejection rates of between the two groups. These results indicate that the higher mating success of older males is attributable to the increased frequency of courtship display rather than a female preference for older males. Moreover, we found that older males produced a larger spermatophore containing more protein than younger males. Older males copulated for a longer period of time than younger males, which probably contributed to the transfer of a larger spermatophore. Although age-related resource allocation in courtship and spermatophore production was consistent with the terminal investment hypothesis, male age did not affect female fecundity and longevity. Deterioration of sperm in the older males might counteract the nutritional benefit of larger spermatophores. Alternatively, fitness components other than longevity and fecundity may be influenced by male age.

Keywords:*terminal investment hypothesis, life-history theory, lepidoptera, courtship display, spermatophore,*

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Ecology

O306

Finding signatures of selection imposed on insect by urbanization

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The process of urbanization is hypothesized to be a new source of selection on the life beings dwelling human-transformed habitats. To test this idea we have chosen two insect species: black garden ant Lasius niger (Hymenoptera: Formicidae) and common house mosquito Culex pipiens (Diptera: Culicidae). Populations of these species from the urban and natural habitat in the same region were sequenced and compared to find the signatures of selection acting on these insects in urban environment. Ant samples are from Moscow region (Russia), and mosquitoes ? from Moscow and Tula region (Russia). Urban samples came from Moscow which is a great megapolis, and from the small industrial town Aleksin (in Tula region) which is less urbanized than Moscow. The natural populations for the comparison were sampled in the rural areas near these cities. The results of the sequencing and theur bioinformatic analysis is upcoming this summer.

Keywords:*urbanization, natural selection, selection signatures*

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Ecology

O306

Mating experience weakens starvation tolerance in the seed bug *Togo hemipterus* (Heteroptera: Lygaeidae)

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Organisms are exposed to various stresses caused by environmental fluctuations. One of the most common stresses is the shortage of food. Individuals of many species must survive periods of starvation. There appears to be a trade-off between reproduction and survival. When residual reproductive value declines for an individual, life-history theory predicts an increase in current reproductive investment. Current reproductive investment differs between virgin and mated individuals. It is likely that mating experience influences starvation tolerance. However, few studies have investigated sex differences in the effect of mating experience on starvation tolerance or clarified the causes of reductions in starvation tolerance in both sexes. In the present study, these questions were investigated using the seed bug Togo hemipterus (Heteroptera: Lygaeidae).The results of the present study demonstrated that mating is costly for both sexes. Mated males showed very short survival times and a daily reduction in weight, and daily energy expenditures were significantly greater in mated males than in virgin males. It is possible that starvation increases the mating effort of males, such as behavioural activities and the amount of time spent searching for females. A trade-off between survival duration and lifetime fecundity was found in virgin females. However, there was no trade-off in mated females, which had very short survival times. Whether male seminal substances contribute to the short survival times of mated females was considered. This is the first report demonstrating the influence of sex and mating experience on starvation tolerance. Sex-specific causes for reductions in starvation tolerance are discussed.

Keywords:*the seed bug Togo hemipterus, starvation tolerance, seminal substances*

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Ecology

O306

Overwintering Adults of *Danaus genutia* (Lepidoptera, Nymphalidae: Danainae) in South-central Yunnan are in quiescence

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Adults of the common tiger butterfly, Danaus genutia Cramer (Lepidoptera, Nymphalidae: Danainae), can be seen all the year round in the river valleys in Yuanjiang county, south-central Yunnan province. The frequency of mated females in the natural population dropped dramatically from early September to middle December with oviposition ceased gradually within this period. From middle November to mid December, no female was found to contain mature oocytes and all males contained undeveloped spermaries. When hold in 27.5℃ (LD16:8) for 10-30 d, 95%-100% of females (n = 15-20) had mature oocytes and all males (n = 35) had developed spermaries. In the laboratory experiments, all females and males kept in 17.5℃ from eclosion had undeveloped ovaries or spermaries, regardless the conditions experienced during larval and pupal stages. After transferred to 25℃ for 10 d, all adults developed mature ovaries or spermaries, with most females containing mature oocytes. The results indicate the reproductive dormancy in overwintering adults is a state of quiescence. We suggest the few (3.45%, n = 58) diapausing females detected might be migrants from the northern distributions.

Keywords:*Danaus genutia, adult, overwinter, quiescence*

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O306F21

Ecology

O306

Characterization of overwintering sites of brown marmorated stink bug (Hemiptera: Pentatomidae) in natural landscape

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It is well-established that adult Halyomorpha halys (Hemiptera: Pentatomidae) overwinter in human-made structures. However, little is known about overwintering ecology of this insect in the natural landscape. In a study spanning seven months from autumn to spring, we assessed 1,400 dead trees and 128 leaf litter samples to locate overwintering H. halys. Samples were taken in the two locations: small woodlots bordering agricultural production areas and large wooded tracts in mountainous areas. For dead trees, crevices under the tree bark were examined up to 3m above the ground. For leaf litter, samples were taken from 1 × 1m areas of the forest floor. In the dead trees, overwintering adult H. halys were found in nearly 4.0% of all trees examined. The mean number of adults per tree was 4.88 (± 2.25). Common traits of dead trees harboring overwintering H. halys adults included: 1) trunks with an upright or standing orientation, 2) oak or locust tree species, and 3) trees with a circumference > 60cm at chest height. Our results indicate that 11% of dead trees in the surveyed woodlots share all three characteristics and therefore could serve as overwintering harborages for H. halys. In the leaf litter, H. halys was never found, whereas native stink bugs including Chinavia hilare, Euschistus servus, and Menecles insertus were present in 16% of samples. The implication of H. halys populations overwintering in the natural landscape is discussed in the context of improving management programs for this invasive species.

Keywords:*Overwintering, Brown marmorated stink bug, Halyomorpha halys*

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Ecology

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The mechanism of the fecundity reduction in *Callosobruchus maculatus* caused by *Callosobruchus chinensis* males

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Recent studies have reported that reproductive interference, or any kind of interspecific sexual interaction which reduces fitness of individuals involved, plays a crucial role in nature causing exclusive relationships between closely related species. However, our understanding of the mechanisms generating reproductive interference is still insufficient. Two congeneric bean weevils, Callosobruchus chinensis and C. maculatus, show asymmetric reproductive interference. Callosobruchus chinensis males reduce the fecundity of C. maculatus, but not vise versa. Here we investigated the mechanism of reproductive interference by C. chinensis males on C. maculatus females in terms of lifetime fecundity. Callosobruchus chinensis males with ablated genitals, which could harass C. maculatus females but not copulate with them, did not reduce the C. maculatus fecundity, suggesting that interspecific copulation was necessary. However, a single interspecific copulation did not affect C. maculatus fecundity as long as the females also copulated with a conspecific male. Exposure to C. chinensis males for 24 h prior to oviposition significantly reduced C. maculatus fecundity, and fecundity was negatively correlated with the number of C. chinensis males the females were exposed to. Moreover, C. maculatus females experienced more interspecific copulations when they were housed with more C. chinensis males. These findings suggest that multiple interspecific copulations by C. chinensis males reduce the fecundity of C. maculatus females. In general, even if a single interspecific copulation is apparently harmless, repeated interspecific copulations can be costly for the individuals involved. Furthermore, only by quantifying reproductive success were we able to identify the precise mechanism of reproductive interference.

Keywords:*interspecific copulation, Callosobruchus, reproductive interference*

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Ecology

O306

Size-induced reproductive constraints in an egg parasitoid

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The size of adult parasitoids is influenced by the quantity and quality of resources available during immature development. Gregarious development of endoparasitoids results in scramble competition where the resources are shared among individuals developing within the same host. Individuals that developed gregariously are therefore smaller and that reduced size generally translates into lower fitness due to reductions in several life history traits including fecundity, longevity and mobility. This phenotypic plasticity in size also affects reproductive traits in parasitoids. We measured the reproductive constraints induced by size in Anaphes listronoti (Hymenoptera: Mymaridae), a facultative gregarious egg parasitoid of Curculionidae. Size decreased with number of immatures developing in a host egg for both male and female A. listronoti. This reduction in size resulted in fewer sperm being produced by males and fewer oocytes by females. Contrarily to other egg parasitoids, A. listronoti males are not prospermatogenic and produced some sperm during their adult life. Females that developed singly received a full sperm complement only when mated by a large male that also developed singly. When mating with a small male, a large female received less than half her sperm complement. Large males ejaculated about 50 sperm per mating and were able to mate four females before the number of sperm transfered started to decrease. When estimating fitness cost of gregarious development, it is important to take into account the reduced fertility of both males and females.

Keywords:*phenotypic plasticity, reproduction, fitness*

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O306F24

Ecology

O306

Influence of "*Candidatus Liberibacter asiaticus*" on the biological characteristics of *Diaphorina citri* Kuwayama

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Huanglongbing (HLB) is the most serious citrus disease. "Candidatus Liberibacter asiaticus" (Las) is one of the 3 Liberibacter species associated with HLB. Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is the only known vector of Las. In this study, the biological characteristics of D. citri feeding on HLB-infected and healthy citrus were compared. The result showed that hosts affected by HLB had different influence on various life stages of the psyllids. The duration of eggs and 1-3 instars, survival rate of 1-2 instars, and sex ratio were not significantly different. Yet, for the former, the duration of 4-5 instars, total nymphs, pre-oviposition of females and the whole generation were significantly shortened; the fecundity and the period of adult laying eggs were significantly increased, and the survival rate of 3rd -5th instar nymths and generation were also significantly increased. The sex ratio of their offspring had no significant difference when feeding on both hosts. Although the egg hatching rate was significantly decreased, the Indice of Population Trend of the psyllids feeding on HLB-infected citrus was significantly higher than that on healthy citrus. These data indicated that infection of Las benefited the development of psyllid population. But, the tolerance to starvation of psyllids feeding on HLB affected hosts was significantly lower than those of feeding on healthy hosts. The mortality rate of overwintering adults feeding on HLB hosts was also significantly higher than those feeding on healthy plants. The detection rate of Las in the psyllids feeding on HLB hosts was 75%.

Keywords:*Diaphorina citri Kuwayama, Candidatus Liberobacter asiaticus, biological characteristics*

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O306F25

Ecology

O306

Thermal biology and population abundance of Mediterranean and Natal fruit flies, *Ceratitis capitata* and *Ceratitis rosa* (Diptera: Tephritidae) in South Africa

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Understanding the link between field thermal biology and insect population dynamics under changing thermal conditions is of fundamental and applied importance and has significant implications for forecasting efforts. Here, we investigate several aspects of thermal biology and population dynamics in two pest fruit fly species, Ceratitis capitata and C. rosa, in South Africa both under laboratory and field conditions. Data on thermal limits across several life-stages, coupled with high resolution climate recordings, showed that upper and lower critical or lethal temperatures are seldom encountered in the field. Trap monitoring found that flies are active all year round, but abundance declines markedly during winter. Microclimate temperatures showed that the frequency and duration of suboptimal low temperatures e.g. lower developmental threshold is high and likely to suppress adult activity, mating, oviposition and development of immature stages. An integrated model that included a range of thermal responses under conditions that compromise development and survival was developed and used to predict changes in population abundance accurately. This model is of broad applicability to fruit fly management globally. These results suggest post-harvest orchard sanitation and adoption of an area-wide approach to fruit fly management may be critical for long-term reductions of Ceratitis populations in the region. This work provides an empirical framework for understanding the relationship between field thermal biology and population ecology of Ceratitis in agro-ecosystems, which is critical for the effective management of these flies.

Keywords:*Ceratitis, thermal tolerance, population dynamics, pest management*

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Ecology

O306

Comparative population growth of the Psocid *Liposcelis yunnaniensis* (Psocoptera: Liposcelididae) on different humidities

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We investigated the effect of four different humidities on population growth of the psocid Liposcelis yunnaniensis (Psocoptera: Liposcelididae). The four different humidities used were; 43, 55, 63, and 75% respectively. In a 30 d period the initial four females psocids increased to the mean populations of 24 ± 5.72 on 75% humidity and 21 ± 3.45 on 63 % humidity. The psocids developing on 43 and 55 % showed no increase in their population and all the adult females psocids died in them. The results show that population growth was highest on higher humidities and the lower humidities of 43 and 55 % lead to the death of the insects. According to the analysis of variance there was no significant difference in the population growth between the higher humidities of 75% and 63% R.H. An analysis was also done with respect to the psocids placement in 4 different compartments of the incubator from top to bottom. It was found that there was no statistical difference in population growth of the psocids in different chambers of the desiccators which could be due to any variation in temperature inside the incubator. Psocid population growth is explained graphically with respect to different humidities and its placement on different heights of incubator.

Keywords:*Relative Humidity, Population Growth, Stored Product Psocids, Liposcelis management*

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O306F27

Ecology

O306

Life tables and development of *Musca domestica* (Diptera: Muscidae) on three different diets

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The life history of Musca domestica reared on three diets (standard laboratory diet, SLD; solid waste diet, SWD; poultry manure diet, PMD) with additional 10% sucrose solution for the adults was studied based on the age-stage, two-sex life table considering both sexes and variable development rates among individuals. The study was conducted at laboratory conditions of 25 ± 2.2 ºC, 60 ± 5% RH and 12:12 h (L:D). Flies on SWD had shorter pupal duration (4.97 ± 0.07 days), shorter total preoviposition period (14.76 ± 0.18 days) and higher fecundity (401.31 ± 3.32 eggs per female) indicating that flies fed this diet were biologically more robust than those on SLD and PMD ($p < 0.05$). Longevity of females on SWD was the lowest possibly due to negative correlation between longevity and fecundity ($p < 0.01$). Overall, the population parameters indicate that M. domestica are r-strategists. Higher intrinsic rate of increase (0.2680 ± 0.0071 days-1), finite rate of increase (1.3073 ± 0.0093 days-1) and net reproductive rate (168.6 ± 20.0 offspring per individual) and shorter mean generation time (19.2 ± 0.2 days) of flies on SWD compared to SLD and PMD ($p < 0.05$) shows the greater nutritional suitability of SWD and potential for population growth. Age-stage specific survival (sxj), life expectancy (exj) and reproductive value (vxj) curves show life stage overlapping due to variation in development rates among individuals.

Keywords:*Life table, development, Musca domestica, diets*

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O306F28

Ecology

O306

A comparison of red palm mite populations on banana in the invasive and naturalised range.

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The Red Palm Mite (RPM) Raoiella indica (Acari:Tenuipalpidae) was first reported in the Caribbean in 2004 and has rapidly spread throughout the region and also into South America and Southern Florida. The mite was first reported in the Old World, in India (Hirst 1924) on coconut and its reported host range there prior to the invasion was relatively small, including: Coconut (Cocos nucifera), Date palm (Phoenix dactylifera) and Betelnut (Areca catechu). In the New World, high populations have been reported on many alternative hosts including the economically important banana, which remained unreported as an Old World host in the literature prior to 2010. To investigate why differences in RPM host range are being reported between the invasive and naturalised range, a series of studies were undertaken on banana. Survival analyses were conducted on local banana cultivars in India to establish whether banana is a potential host there. These indicated that RPM was able to survive and lay eggs on banana leaf sections thus having the potential for population establishment in the field, although performance differed between cultivars. A field survey was also undertaken in Kerala, India and Trinidad to estimate RPM and predator densities on banana, which indicated that predator densities and diversity were much higher in India on banana suggesting natural control of populations by predators on banana in this region. A simple population model developed from survival analysis results supported these findings.

Keywords:*Raoiella indica, Red Palm Mite, banana*

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O306F29

Ecology

O306

Comparative life history and life tables between the two clones of *Sitobion avenae* (Fabricious) (Hemiptera: Aphididae) on barley yellow dwarf wirus infected barley

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Life history and life tables of the brown and green clones of the apterous grain aphid, Sitobion avenae (Fabricius), were examined on barley yellow dwarf virus (BYDV) strain PAV infected, virus-free aphid pre-infested and on non-infected barley plants under the laboratory conditions of 20±1ºC, 65±5% RH and a photoperiod of 16:8 (L: D) h. Generally, both of the two S. avenae clones performed significantly better when fed on BYDV-infected plants compared with those fed on non-infected or virus-free aphid pre-infested plants. Furthermore, when fed on BYDV-infected plants, the green S. avenae clone developed significantly faster and had a significantly longer reproductive period compared with those of the brown clone. No significant differences were found for either clone of S. avenae when fed on non-infected or virus-free aphid pre-infested plants. The results showed that BYDV positively affected the performance of the two S. avenae clones, and particularly the green clone.

Keywords:*Sitobion avenae, color morph, biology, Barley Yellow Dwarf Virus*

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O306F30

Ecology

O306

The glassywinged sharpshooter, *Homalodisca vitripennis* and the plant pathogenic bacterium, *Xylella fastidiosa* it vectors: Preparing Australia for invasion

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The glassy-winged sharpshooter, Homalodisca vitripennis Germar (Hemiptera: Cicadellidae), is an important insect vector of the xylem-limited plant pathogen Xylella fastidiosa Wells which causes diseases in numerous plant species including food and feed stock crops, ornamentals and weeds. Both the pathogen and the vector are native to the Americas and are considered to be highly invasive but, to date, neither have been detected in Australia. Past invasions of the bacterium and vector have demonstrated the need for rapid detection and containment of an incursion. This requires a detailed knowledge of potential H. vitripennis and X. fastidiosa interactions and host plants in the invaded range. These host plant species can then be targeted for monitoring in order to detect an incursion at an early stage. Australian native plants naturalised in California provided an ideal opportunity to explore how the pathogen and vector, both also present in California, would interact with these plants if they were to arrive in Australia. Twelve Australian native plant species of interest were assessed as hosts of the pathogen and vector using field, greenhouse and laboratory studies in California, USA. Additional studies were undertaken to determine the risk of air transport as an invasion pathway, and the activity of the native enemy of H. vitripennis, parasitoid wasps, on Australian native plants. The results of the host status, invasion pathway and natural enemy research are presented and the implications of these findings discussed and placed in an Australian invasion context.

Keywords:*Biosecurity, Pierce's disease, invasive insect, exotic plant disease*

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O306F31

Ecology

O306

Difference in larval development and female oviposition preference in host plants between two populations utilizing different plants as larval hosts in an endangered lycaenid butterfly, *Zizina emelina*

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The bird's-foot trefoil, Lotus japonicus (Lj) (Fabaceae), has been known as a primary host plant of an endangered lycaenid butterfly, Zizina emelina. In the Kinki district, central Japan, a population in Kato City (KT), Hyogo Prefecture, utilizes Lj as a larval host, but a population depending on the white clover, Trifolium repens (Tr) (Fabaceae), was recently found in Toyonaka City (TN), Osaka Prefecture. In this study, we compared development of larvae fed with each of the two plants and oviposition preference of the resulting females in host plants in progenies of all combinations between KT and TN (all progenies). As for the larval development, the pupal weights of TN were about 51 and 48 mg in median in groups fed with Lj and Tr, respectively, and larger than those of KT (47 and 39). In all progenies the pupal weights of Lj fed groups were larger than those of Tr fed groups. Larval survival rates were not significantly different in all progenies fed with different host plants. Adult females of Lj and Tr fed groups in TN laid 30.9 and 42.1% eggs on Tr, respectively, while those in KT oviposited very few eggs (0.8 and 0.0%) on Tr. The pupal weight and oviposition preference of the hybrids were intermediate between those of TN and KT. The number of eggs laid per female was correlated with the pupal weight. The results show that females of TN lay more eggs and larval development is better on Tr than those of KT.

Keywords:*larval development, oviposition preference, host plant, Zizina emelina, Japan*

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O306F32

Ecology

O306

Effect of host plant on life history and population growth parameters of the invasive mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) in Africa

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The effect of four cultivated host plants namely Mangifera indica L., Cajanus cajan (L.) Millspaugh, Coffee arabica L. and Cucurbita moschata Duchesne, and two important ornamental plants namely Parkinsonia aculeata L. and Ficus benjamina Roxb., on the biology of the mango mealybug, Rastrococcus iceryoides Green (Hemiptera: Pseudococcidae) was studied in screen houses at 22.3 ± 5.07˚C, 70 ± 5% relative humidity and 12L:12D photoperiod. The development, survivorship, longevity, reproduction and life table parameters of R. iceryoides differed significantly among the plant species. The shortest developmental period (from egg to adult) was recorded on M. indica (23.5 ± 0.34 days for females) whereas the longest was recorded on F. benjamina (33.0 ± 0.62 days). The highest egg to adult female R. iceryoides survivorship was recorded on C. moschata (79.6 ± 1.4%) and the lowest was on C. arabica (30.9 ± 1.1%). The highest fecundity was observed on C. moschata (811.3 ± 7.3 eggs). The sex ratio was female-biased on C. moschata, M. indica, C. cajan and P. aculeata. Body size was influenced by the different host plant types. Maximum values of the intrinsic rate of natural increase (rm), finite rate of increase (λ) and the shortest mean generation time (GT) and doubling time (Td) were recorded on M. indica. This implies that M. indica is the most preferred compared to the other host plants. Results of this study are useful in understanding the roles of host plants in integrated management of this pest, including exploitation of these plants in push-pull control.

Keywords:*Rastrococcus iceryoides, Host plants, Development, Survivorship, Reproduction*

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Ecology

O306

Modeling temperature-dependent development of pistachio psylla, *Agonoscena pistaciae* (Hemiptera: Psyllidae)

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The pistachio psylla, Agonoscena pistaciae Burckhardt & Lauterer (Hemiptera: Psyllidae) is a major pest of pistachio trees throughout pistachio producing regions in Iran. The objective of this work was to evaluate which linear and nonlinear model, (Stinner aplo, Janisch, Taylor, Sharpe & DeMichele, Lamb, Lactin and Briere) best describes the relationship between developmental rates of the egg, nymph and egg to adult of A. pistaciae, and temperature. The pistachio psylla eggs and nymphs were reared on pistachio leaves (Pistacia vera L., cultivar Ohadi) at constant temperatures of 15, 20, 25, 30, 32.5 and 35±0.5ºC. The models were evaluated based on four indicators; (R2, RSS, AIC and R2adj). For egg, the linear model and nonlinear models, Stinneraplo, Lamb, Taylor, Sharp & DeMichele and Lactin, appropriately described the relationship between the developmental rate and temperature. For nymph and egg to adult, the nonlinear models are not appropriate to describe the observed data accurately. Using the linear model the lower temperature threshold for egg, nymph and egg to adult was estimated 8.06, 10.38 and 9.97ºC, respectively. The similar value of lower temperature threshold was estimated by Lactin model especially for egg (8ºC). Thermal constant for egg, nymph and egg to adult development by linear model was estimated 88.5, 243.90 and 333.33 DD, respectively. Our results showed that, the linear model better describe the relationship between developmental rate and temperature of A. pistaciae. These results could be incorporated in forecasting models for integrated pest management of this pest.

Keywords:*Agonoscena pistaciae, Critical temperature, Developmental rate, Modeling*

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O306F34

Ecology

O306

Multiple mating and its effect on fitness of *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

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Mating frequency and the amount of sperm transferred during mating have important consequences on progeny sex ratio and fitness of haplodiploid insects. Production of female offspring depends primarily upon the availability of sperm for fertilising eggs. This study examined multiple mating and its effect on fitness of the cabbage aphid parasitoid Diaeretiella rapae (Hymenoptera: Aphidiidae). Female D. rapae mated once, while males mated with on average more than 3 females in a single day. The minimum time lag between two consecutive matings by a male was 3 min, and the maximum number of matings a male achieved in a day was 8. Sperm depletion occurred as a consequence of multiple mating in D. rapae. The number of daughters produced by females that mated with multiple-mated males was negatively correlated with the number of matings achieved by these males. Similarly, the proportion of female progeny decreased in females that mated with males that had already mated 3 times. Although the proportion of female progeny resulting from multiple mating decreased, the decrease was quicker when the mating occurred the same day than when the matings occurred once per day over several days. Mating success of males initially increased after the first mating then decreased with the number of prior matings. The fertility and the progeny sex ratio of females were affected by mating with multiple mated males. Study suggests that male mating history affects the fitness of male and female D. rapae.

Keywords:*Diaeretiella rapae, multiple mating, sperm depletion, sex ratio*

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O401M01

IPM

O401

Invasive insect pests of forests in the Southern Appalachians, USA: Assessment and management of multiple pests to enhance forest health

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Multiple introduced and invasive exotic insects, weeds, and plant diseases currently impact forest health in the southeastern United States, where forests are comprised of numerous tree species. Three of these species, ash (Fraxinus spp.), black walnut (Juglans nigra), and eastern hemlock (Tsuga canadensis), are currently threatened by invasive insect pests. The hemlock woolly adelgid, Adelges tsugae, has been present on hemlocks in southern forests since 2002 and has killed tens of thousands of eastern hemlock trees. In 2010, the emerald ash borer and thousand cankers disease (TCD) (caused by a fungal pathogen, Geosmithia morbida, vectored by the walnut twig beetle, Pityophthorus juglandis) were found on ash and black walnut trees, respectively, in the southern United States. Although TCD has not yet been recovered from a forest setting, its documentation in urban habitats was the first comfirmation of TCD in the native range of black walnut and the first incidence of either pest problem in the southern United States. The presence of emerald ash borer in Tennessee represents the most southern distribution of this invasive pest in the U.S. The presence of emerald ash borer and TCD in Tennessee provides a tremendous opportunity to expand research and outreach activities to better understand these invasive organisms in the southern United States. Knowledge gained here can be applied throughout the native range of black walnut to enable scientists to develop better management strategies to limit the impact of this devastating disease and to enhance management efforts against emerald ash borer in southern areas.

Keywords:*hemlock woolly adelgid, thousand cankers disease, emerald ash borer, forest health, biological contr*

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O401M02

IPM

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Biological base of IPM against phytophages on strawberry plantations

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IPM against a number of insects (mainly strawberry blossom weevil Anthonomus rubi Herbst, strawberry leaf-roller Ancylis comptana Frol.) and mites (mainly Tetranychus atlanticus McGregor) on strawberry plantations in the Central Russia has been constructed on the base of long-term observations. Life tables, phenology, plant injury calculation methods were used. Phytophages monitoring was carried out on each plot of 2-2.5 hectares. Main insect control criteria included thresholds of harmfulness (for A. rubi ? 0.1-0.25 beetle, for A. comptana ? 8-12 caterpillars per 1 linear m of row at the period of first visible flower buds), for spider mite control ? prevention the population density of 35-40 individuals (excluding eggs) per 1 leaflet at fruit formation period. The threshold of harmfulness of A. rubi on tolerant strawberry varieties could be increased to 0.66 beetles per 1 linear m. Outbreaks of spider mite populations were expected when the density of overwintered females was 0.4 and more per 1 leaf and when the mean decade temperature at each of 4-5 following decades reached or could be above 13.5-14°C. In these years, the net reproduction rate (R0) of the overwintered population was 46-65, and that of the first summer generation reached 80-95. When the economic level of spider mite damage was expected at the period of fruit formation, one of the predatory mite species of Neoseiulus was used at the period of blooming. In some years, we could exclude chemical treatments on the half of the 200 hectares of strawberry plantations.

Keywords:*IPM, phytophages, Anthonomus rubi, Ancylis comptana, spider mites, strawberry*

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O401M03

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O401

Synthetic substrate-borne vibrational signals that elicit Asian citrus Psyllid communicatory and search responses

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The Asian Citrus Psyllid (ACP), Diaphorina citri Kuwayama, vectors a harmful bacterium, Candidatus Liberibacter asiaticus, that causes huanglongbing, an economically devastating disease of citrus. Adult male and female ACP transmit vibratory communication signals over 10-50-cm distances within their citrus tree hosts by producing wing-fanning vibrations. Males begin calling while searching for females. When a receptive female detects these signals, she replies in a duet that facilitates location of her position. This report describes explorations of new methods that use such signals to efficiently detect and trap ACP males for facilitation of targeting and control efforts. In preliminary studies, mating communication signals of virgin adult male/female pairs were recorded on citrus seedlings at 3-7 days post eclosion. Candidate signals were played back to elicit responses from other psyllids on new seedlings. A series of synthetic calls was constructed, based on the mean durations and spectral harmonics of male and female signals. The synthetic calls were tested using a vibration exciter attached to the host tree. The elicited behaviors were monitored and recorded. The results were compared to each other, as well as to results from playbacks of recordings from males and females. In general, the more harmonics that were present in the synthetic song, the greater the likelihood that a female would reply or that the male would orient to the signal. A goal of future studies is to devise an inexpensive, efficient trap for males, and ultimately to broadcast synthetic signals that can be used to interfere with mating.

Keywords:*attraction*

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Assessment of the effect of inflorescence density index of grain amaranth (*Amaranthus* Spp.) on *cletus fuscescens* infestation.

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ABSTRACT. The effect of inflorescence density index of grain amaranth on Cletus fuscescens attack was studied during the early seasons of 2009 and 2010 in the experimental field of National Horticultural Research Institute, Ibadan. Accessions tested were 'RRC 646', 'P 373' and Oscar Blanco, (all with lax inflorescence density index), 'RRC 1027', 'Tibet' and Niqua, (all with intermediate inflorescence density index) and 'D70-1', 'RRC 551' and Zhen Ping, (all with dense inflorescence density index). All the 3 accessions with intermediate inflorescence density index had highest Cletus fuscescens populations/ plant and least viability percentage while all the 3 accessions with dense inflorescence density index had lowest C. fuscescens populations/ plant and highest viability percentage. C. fuscescens populations/ plant established a strong negative relationship with only viability percentage. Also stepwise regression analysis selected viability percentage as the most powerful variable reflecting C. fuscescens infestation. This result suggests that dense inflorescence density index in grain amaranth contributed to reduction in bug infestation which indicates high seed viability percentage and grain quality.

Keywords:*Cletus fuscescens, inflorescence density index, grain amaranth, seed viability.*

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O401M05

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O401

Management strategies for the invasive fruit fly, *Bactrocera invadens* in Africa

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Bactrocera invadens (Diptera: Tephritidae) is a recently described fruit fly species of major quarantine concern. It attacks a variety of fruits and vegetables but mango is the preferred host plant. We evaluated various synthetic food-based attractants that could be used in combination with entomopathogenic fungi (EPF) against adults and for soil inoculation targeting pupariating larvae and puparia. The field performance of Mazoferm and GF-120 spinosad bait spray in combination with Metarhizium anisopliae-based biopesticide will be presented. Since native fruit fly parasitoids do not play an important role in the management of B. invadens, exploration for natural enemies of the pest in its putative aboriginal home of Sri Lanka was undertaken. Exploration yielded a diversity of parasitoids and predators but none has been introduced into Africa for management of B. invadens due to restrictions related to Convention on Biological Diversity. However, two exotic parasitoids (Fopius arisanus and Diachasmimorpha longicaudata) have been introduced from Hawaii for the control of B. invadens in Africa. The potential of these parasitoids in the management of the pest is examined and results from ongoing field release efforts that are complementing other management methods based on baiting and male annihilation technique, use of biopesticides and orchard sanitation for the management of the invasive fruit fly in Africa are presented. In addition to pre-harvest management measures, results will be presented on post-harvest treatment parameters that have been developed to facilitate access to quarantine sensitive markets.

Keywords:*Bactrocera invadens, attractants, entomopathogenic fungi, parasitoids, sanitation, suppression*

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O401M06

IPM

O401

Improved attraction of the strawberry blossom weevil *Anthonomus rubi* by combining the aggregation pheromone with specific host flower volatile

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In the absence of effective control measures, the strawberry blossom weevil (Anthonomus rubi) cause large losses in both conventional and organic grown strawberry in northern and central Europa. The present study aimed to investigate the significance of plant volatiles in combination with the aggregation pheromones to develop an effective semiochemical trap for the management of A. rubi. The aggregation pheromone has been available for several years. However, it has shown low attractancy. In the present study host plant volatiles from wild strawberry (Fragaria vesca) and cultivated strawberry (F. ananassa) were collected by SPME. These volatiles were chemical identified using GC-MS. The volatile blends of F. vesca and F. ananassa were dominated by one and six different compounds respectively. For both plant species other compounds were also identified. To test the significance of the major flower volatile of F. vesca and one other relevant compound from F. ananassa in combination with the aggregation pheromone field experiments were established in Norway, Denmark and UK. Green funnel traps with white cross veins were used for the evaluations. The results shows that the traps baited with plant volatiles didn't attract more A. rubi than control traps without baits. However, traps baited with the major flower volatile from wild strawberry flowers and the aggregation pheromone increased trap catches by over two fold compared to the pheromone alone. Thus, the combined use of the aggregation pheromone and this flower volatile is promising for improved monitoring and possibly control of this important pest of strawberry.

Keywords:*Anthonomus rubi, Fragaria sp., aggregation pheromones, plant volatiles, insect control*

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O401M07

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O401

Stylet penetration activities of tomato/potato Psyllid associated with acquisition and inoculation of *Candidatus Liberibacter solanacearum*

Manoharie Sandanayaka[1], Aranzazu Moreno[2], Leah Tooman[3], Natalie Page-Weir[4], Alberto Fereres[5]

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The stylet penetration activities relating to acquisition and inoculation of Candidatus Liberibacter Solanacearum (CLso) by a single Tomato/Potato Psyllid (TPP), Bactericera cockerelli, on tomato were studied using electrical penetration graph (EPG-DC system) recordings. Female TPP from CLso-positive and negative colonies were used in inoculation and acquisition tests, respectively. The presence or absence of CLso in positive and negative colonies, donor plants used in acquisition tests, plant material after inoculation tests and insects after EPG tests, was confirmed by PCR tests. In the acquisition tests, the stylet penetration activities of 70 TPP were monitored on excised shoots of CLso-positive tomato plants for 4-24 hours. In the inoculation tests, the stylet penetration activities of 60 individual TPP were monitored on 60 healthy (CLso free) tomato plants for 12-24 hours. Durations of EPG waveforms (C, G, D, E1 and E2) representing the main stylet penetration activities of individual insects in different acquisition and inoculation access periods were measured. Results confirmed that a single adult TPP can inoculate CLso and cause damage to a plant. The relationship between EPG waveforms and PCR results suggest that acquisition occurred during phloem ingestion (E2) and inoculation was preferentially associated with salivation into the phloem sieve elements (E1). The minimum plant access period required for CLso transmission by a single TPP was estimated, to assist growers in making effective management decisions to reduce the impact of this serious disease.

Keywords:*Bactericera cockerelli*

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O401M08

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O401

Oviposition preferences of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and an oviposition preference-based susceptibility index for apple cultivars

Neelendra K. Joshi[1], Larry A. Hull[2], Edwin G. Rajotte[3], Clayton T. Myers[4], Greg Krawczyk[5]

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Oviposition preferences of the codling moth (CM), Cydia pomonella (L.) (Lepidoptera: Tortricidae) for the fruits of different apple cultivars were studied in laboratory bioassays using a series of no-choice and multiple-choice tests. During the first two years of study, ten apple cultivars viz., Arlet, Fuji, Gala, Golden Delicious, Honeycrisp, Pristine, Delicious, Stayman, Sunrise, and York Imperial were evaluated, while in the third year, Golden Delicious, Honeycrisp and York Imperial were evaluated. In the first year's tests, preferred apple cultivars for CM oviposition were Golden Delicious and Fuji, while the least preferred were Arlet, Pristine, Sunrise, and Honeycrisp. Similarly, during the second year, Golden Delicious, Fuji and Stayman remained the preferred cultivars, while Arlet, Honeycrisp, Pristine, and Sunrise remained the least preferred cultivars. In the third year's tests, Golden Delicious and Honeycrisp were the most and least preferred cultivars, respectively. Based on the oviposition preferences from these bioassays, a CM oviposition susceptibility index for each cultivar was developed. This index may be used as a standard measure in cultivar evaluations and may assist fruit growers and consultants to select the most appropriate cultivar(s) for monitoring and detecting the initial signs of fruit injury from this pest in an apple orchard planted with mix-cultivars. Oviposition in CM is likely stimulated by fruit volatiles, and variations in the production and release of these volatiles from different apple cultivars may result in different oviposition preferences. Further investigations are needed to understand biochemical aspects and other factors involved in determining the oviposition preference of CM.

Keywords:*Cydia pomonella, apple cultivars, oviposition preference, susceptibility index*

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O401M09

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Effects of electron beam on life stages and DNA damage of American serpentine leafminer, *Liriomyza trifolii* (Burgess)

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The American serpentine leafminer fly, Liriomyza trifolii (Burgess), is one of the most destructive polyphagous pests worldwide. In this study, we determined electron beam doses for inhibition of normal development of the leaf miner and investigated the effect of electron beam irradiation on DNA damage and p53 stability. Eggs (0 - 24 h old), larvae (2nd instar), puparia (0 - 24 h old after pupariation) and adults (24 h after emergence) were irradiated with increasing doses of electron beam irradiation (six levels between 30 and 200 Gy). At 150 Gy, the number of adults that developed from irradiated eggs, larvae and puparia was lower than in the untreated control. Fecundity and egg hatchability decreased depending on the doses applied. Reciprocal crosses between irradiated and unirradiated flies demonstrated that males were more radiotolerant than females. Adult longevity was not affected in all stages. The levels of DNA damage in L. trifolii adults were evaluated using the alkaline comet assay. Our results indicate that electron beam irradiation increased levels of DNA damage in a dose-dependent manner. Moreover, low doses of electron beam irradiation led to the rapid appearance of p53 protein within 6 h; however, it decreased after exposure to high doses (150 Gy and 200 Gy). These results suggest that electron beam irradiation induced not only abnormal development and reproduction but also p53 stability caused by DNA damage in L. trifolii. We conclude that a minimum dose of 150 Gy should be sufficient for female sterilization of L. trifolii.

Keywords:*Electron beam, Liriomyza trifolii, Sterility, DNA damage*

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O401M10

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O401

Lac insects cultivation, ecological and economical importance of lac agroecosystems in China.

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Lac is a layer of red resin on branches of host-trees on which lac insects settle. Lac resin is natural, biodegradable and non-toxic, and thus widely used in food, textiles and pharmaceutical industries in addition to surface coating, electrical component manufacturing, and other fields. Several lac insect species and many species of plant trees were collected around the world in China. Some lac insect species have been used to produce lac after many years' domestication. To increase lac yield, the ecological and biological features of lac insects including the relationship between lac insects and host plants, the ways of controlling pests of lac insects and so on were focused on by many researches. The current prosperity in the lac market provides an incentive for farmers to increase lac production. Lac-production and farming were integrated into an agroforest system. Farmers there enjoyed both cash income and non-cash benefits from these multipurpose lac plants, especially the increased soil fertility and soil retention, with limited labor input, in that fragile environment. Under a diverse and thin canopy of lac plants with lac insects, the compounded system harbored a high biodiversity of associated organisms. During the past fifty years, this traditional farming system has been modified by agricultural conversion. Lac systems have either intensified into other food crop agricultural systems following habitat clearing, or abandoned resulting in natural afforestation. Here we illustrate the ecological and economical importance of these agroecosystems, and hope to attract more attention to this valuable agroecosystem.

Keywords:*agroecosystem, biodiversity, ecological-economy, lac insects, host pants,*

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Going underground - enhanced detection approaches for root feeding grape phylloxera

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Grape phylloxera (Daktulosphaira vitifoliae Fitch) is present in most grape-growing countries and can attack both the roots and foliage of grapevines, depending on the genetic lineage of the insect. The root-galling form of phylloxera causes significant economic damage to European grapevine (Vitis vinifera L.) and can also survive on the roots of 'resistant' Vitis rootstocks. Phylloxera detection primarily still relies on visual examination of the grapevine root system and relies on detecting 'weak' spots in the grapevine canopy caused by extensive root damage which often takes years to become evident. Early detection of root feeding phylloxera needs to consider a more targeted approach where the influence of soil physiochemical properties on phylloxera establishment and subsequent dispersal are recognised and where the host plant response, in the form of changes in host plant chemistry, can be used as a secondary indicator of root feeding. Novel options for targeted early detection of phylloxera are being developed, where soil factors are considered, include the use of a soil molecular DNA probe, trapping techniques and soil electromagnetic induction surveys. These techniques can also be combined with the use of spectrochemical sensing to enhance sensitivity. An overview of a novel early detection system under development in Australia will be presented.

Keywords:*phylloxera, Vitis, detection, surveillance, fingerprinting, chemical, molecular, remote sensing*

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Cockchafer distribution, species diversity and seasonal abundance in Gippsland, Victoria: Preliminary findings

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Red Headed Cockchafer (RHC) Adoryphorus couloni (Bermeister) is a native pest of ryegrass-based cattle pastures in South Eastern Australia. Detection is often only after extensive plant damage occurs, with the predominant management technique then being to remove and re-sow pasture. Anecdotal evidence suggests that soil characteristics, vegetation and moisture may influence RHC distribution and population density, though few studies have been conducted to qualify this influence. Other cockchafers such as African Black Beetle Heteronychus arator and Black Headed Cockchafer Acrossidius tasmaniae (Aphodius tasmaniae) may occur simultaneously, and the influence of soil characteristics on their distribution is also unknown. Understanding this influence on cockchafer distribution could lead to improved early detection and targeted management of these pests. In a preliminary 2011 study three regions in Gippsland, Victoria were surveyed using manual soil sampling and on-the-go proximal sensing techniques to (i) investigate any association between soil electrical conductivity (a widely-used surrogate indicator of soil texture, moisture content and/or salinity) and above-ground pasture biomass and the spatial distribution of cockchafer in dairy paddocks, (ii) to quantify the relative abundance of each species in the three regions and (iii) collect preliminary data to determine seasonal distribution of cockchafer life-stages in each region. Results indicated that not all species were found together in all regions, and that a potential link between soil characteristics and cockchafer distribution may exist. It also highlighted that detailed population dynamics studies are a fundamental prerequisite for optimising control on-farm. This research forms part of an ongoing 3 year study (2011-2014)

Keywords:*cockchafer, detection, distribution, soil sensing*

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Grapevine phylloxera: An integrated approach to management of a phylloxera clonal lineages in Australia

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Grapevine phylloxera Daktulosphaira vitifoliae Fitch attacks the roots and leaves of commercial and ornamental grapevines and has spread to most grape-growing countries. It was first detected in Australia in 1877 and is currently restricted to areas in Victoria and New South Wales primarily through quarantine and the use of resistant rootstocks. Phylloxera 'outbreaks' have been increasing in Australia in the last decade. To restrict spread of the insect an integrated approach to management is under development which considers critical stages of the insects' life cycle and three phases of management (i) detection and surveillance (ii) screening for resistant host plant material (iii) quarantine and disinfestation procedures and (iv) grower awareness. All three management approaches are influenced by the diversity and virulence of phylloxera clonal lineages. To develop resilient rootstock resistance ratings a triphasic approach, using laboratory, glasshouse and field protocols, was conducted using six phylloxera clonal lineages and ten rootstock types. The efficacy of chemical and physical disinfestation treatments for viticultural machinery and grape products and the importance of adherence to quarantine protocols is also highlighted. Dry heat treatment proved effective as a disinfestation treatment for first instar grape phylloxera at temperatures of >40 C. Several factors influenced survival of phylloxera in white grape juice including settling temperature and added sulphur. All outputs from research are delivered to growers at annual National Phylloxera Management workshops.

Keywords:*Vitis, Resistance, disinfestation, qurantine, clonal variation*

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Study on occurrence characteristics of *Eucryptorrhynchus chinensis* (Olivier) (Coleptera:Curculioniclae) in Lingwu, Ningxia

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The survey on damage and occurrence characteristics of Eucryptorrhynchus chinensis (Olivier) in Lingwu, Ningxia, revealed that different sites have varied degrees of damage by this pest because of invasive sequence. The damage is most serious in Linhe Town, while less serious in Chongxing Town. E. Chinensis larvae damage the roots of Ailanthus altissima, then stimulate them to form nodules, and lead to fracture of the roots. The adults feed on the exposed roots, the parts with gum or the trunk base,or feed on and wither the shoots. During the recent two years, E. chinensis adults peaked at late June and late August, late May and early July, respectively. About 80% of E. chinensis larvae were found in a soil layer from the surface to as deep as 45 cm underground. The larvae emerge in large numbers in soil from early May to late June. Unearthed extent of most E. chinensis ranges from the base of the trunk to about 75 cm outwards, account for 88% of the total number. Based on damage characteristics of E. chinensis mentioned above, it is practical to catching and killing this pest during its peak. In addition, in chemical control, it may be effective to take into account different orientations and extend the depth and range of the application of pesticide. The optimal time to apply pesticide is May or June, when the larvae occur in large numbers in soil.

Keywords:*Eucryptorrhynchus chinensis, Ailanthus altissima, root, adult occurrence peak, damage*

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Resistance of onion cultivars to thrips (Thysanoptera: Thripidae) and Iris yellow spot virus in Kenya

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Iris yellow spot virus (IYSV) vectored by Thrips tabaci Lindeman, threaten onion (Allium cepa L.) production in Kenya. Insecticides are widely used for vector management resulting in pesticide resistance and increased virus spread. Information on resistance among commonly grown onion cultivars to IYSV and T. tabaci is crucial for the development of integrated virus management strategies, but often lacking. Therefore, this study focused on screening commonly grown onion cultivars (Red pinnoy, Red creole, Bombay red, Green bunching and Texas grano) for thrips and IYSV resistance, in two season field trials conducted at University of Nairobi, Kabete. Each onion variety was replicated four times and arranged in a randomized complete block design. Natural thrips and IYSV infestation occurred and Straw-colored, necrotic lesions typical of IYSV infection was observed two weeks after transplanting. Ten randomly selected plants per plot were rated for IYSV severity and thrips infestation levels. Disease incidence was scored by calculating (number of IYSV infected plants/ total number of plants per plot).The virus titre was determined by DAS-ELISA. Cultivars differed significantly for both disease incidence ($F_{4,44}=5.36$; $P<0.0001$) and thrips infestation levels ($F_{4,44}= 3.67$; $P<0.001$) from fourth week. Red pinnoy, Green bunching and Red creole were more susceptible to the virus and thrips as compared to Texas grano and Bombay red. There were significant differences among cultivars for increase in the virus titre level over time. However, optical densities correlated positively with disease severity ($R=0.663$; $P=0.05$). Texas grano and Bombay red exhibited some tolerance both to thrips and IYSV.

Keywords:*Iris yellow spot virus, Thrips tabaci, Allium cepa, resistance*

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Dispersal and population genetic structure in codling moth at different spatial scales

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Codling moth, Cydia pomonella (Lepidoptera: Tortricidae) is the main pest of pome fruits worldwide. Area wide management strategies of this pest have been used in different countries with different levels of success. Dispersal and gene flow between populations in managed and unmanaged habitats seems to be an important factor to explain such variable results. We used microsatellite molecular markers to estimate codling moth dispersal and gene flow at different spatial scales from country (1800 km) region (250 km) and local scale (< 1 km) in apple producing areas from Chile. In addition, we used immunomarking techniques to estimate dispersal at local scale. We have found low genetic differentiation (Fst) and high gene flow between codling moth populations at different spatial scales, with only slight isolation by distance at larger spatial scales. Bayesian assignment tests also support the low levels of population genetic structure among locations and host-plants. Dispersal studies at local scale using immunomarking techniques support the relevance of adult dispersal to explain the low levels of population differentiation. Anthropic influence on agroecosystems is a main driver of dispersal and gene flow of this pest at different spatial scales.

Keywords:*Cydia pomonella, gene flow, AMOVA*

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Simulation of distributional pattern of leafhopper with GIS

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The evacanthine leafhopper genus, Pagaronia (Hemiptera, Cicadellidae), is distributed in the eastern Palaearctic region and the western Nearctic region, and highly diversified especially in Japan. This high diversification, about 100 species has been described from Japan, is one of interesting evolutionally processes to discuss leafhopper classification. The authors guessed that the allopatric distribution and narrow distribution area of each species should be related to the diversification processes, because these features are not found within the other leafhoppers. Three Pagaronia species distributed in northern Kyushu were used in this study: the distribution of them is completerly allopatric. As the geology, current flora and distribution of large rives in Kyushu were not consistent with the actual distribution pattern of them, two computer simulations with GIS were performed by a Visual Basic Program. A simulation based on the mean annual temperature was not also consistent with the actual distribution pattern. The predicted distributions of them were overlapped. However, a simulation based on the geographical feature, "watershed", was strongly consistent with it. In this simulation, we assumed that leafhoppers can't go over large rivers because they don't fly in middle or long distance. The watersheds between Matsuura River - Rokkaku River, Mikasa River - Chikugo River, and Midori River ? Gokase River are very important for the determination of their distribution.

Keywords:*simulation, distribution, Hemiptera, Cicadellidae, watershed*

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Using thermal tolerance traits to test distribution model outcomes for an invasive mite, *Halotydeus destructor*

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Invasive species cause massive losses to biodiversity and agricultural production globally. Species distribution modelling is often used to predict distributions for invasive species, however, there is little empirical research to test hypotheses generated from these models. The redlegged earth mite, Halotydeus destructor, is native to South Africa and was first reported in Australia in 1917 where it has since established as a major agricultural pest. In the last 40 years this species has expanded into hotter and drier areas. Recently, species distribution models were applied to H. destructor in both its native and invasive ranges and suggested niche-shift to facilitate range expansion for this species. Halotydeus destructor presents a unique opportunity to test species distribution model hypotheses by investigating thermal tolerance traits across a known pathway of invasion and across areas of range expansion. We selected populations of H. destructor from environments in both South Africa and Australia predicted to occupy different niches. Upper activity limits and critical thermal minimum (CTMIN) were tested on field-collected populations of H. destructor. We also tested for effects of acclimation on CTMIN to determine levels of plasticity between native and invasive ranges. Halotydeus destructor populations in Australia have a marginally higher upper activity limit, and are able to recover from CTMIN more rapidly than the South African populations. Australian populations also have higher plasticity than native South African populations. Our results demonstrate that species distribution models provide important foundations to direct explicit testing of traits that may shift between geographic ranges following species invasions.

Keywords:*Species distribution modelling, Thermal tolerance traits, Invasive species*

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Linking vegetation indexes from remote sensing and long-term prospection data to help in the preventative control of desert locust

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Desert Locust management is nowadays done through a preventative control strategy that consists in avoiding populations to reach high densities and a gregarization process leading to uncontrollable swarms. The areas of potential start of the gregarization process for Desert Locust are large and preventative management teams need to prospect all these areas to be efficient. A challenge of ongoing research is to be able to guide on where prospection surveys should be done depending on meteorological and vegetation conditions. An analysis of relationship between long-term prospection data of Desert Locust observations from 2005 to 2009 and spatio-temporal statistics of a vegetation index gathered by remote-sensing was conducted using multiple logistic regressions. The vegetation index was a composite Normalized Difference Vegetation Index (NDVI) given every 16 days and at 250m spatial resolution (MOD13Q1 from MODIS satellite). The statistics extracted from this index were: 1) spatial means at different scales around the prospection point, 2) relative differences of NDVI variation through time before the prospection and 3) large scale summary of vegetation quality. Identical statistics could potentially be computed for actual NDVI. By extrapolation of adequate logistic regression models, maps of probability of presence of locust could be constructed. This methodology should help in focusing prospection toward sensible parts of the gregarization areas at specific times.

Keywords:*Desert locust, remote sensing, population dynamics, logistic regression, vegetation dynamics*

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Comparative study based on arthropods biodiversity indicators on paddy fields between integrated pest management and conventional technologies

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Agricultural ecosystem of paddy is an ecosystem that has a high arthropods biodiversity. Arthropods play the main role to the successfulness of agricultural ecosystem. Population and diversity of arthropods on paddy field is related to agronomic activities. The research was aimed to compare the impact of Integrated Pest Management (IPM) technology and conventional one on the paddy field based on arthropods biodiversity indicators. Research was conducted on paddy field located in Banyuwangi Regency, East Java, Indonesia. Both technologies were separated into two fields. To observe arthropods biodiversity upper ground, pan and pitfall traps were arranged systematically on field. In addition, soil samples were collected to evaluate below ground arthropods. The result showed that arthropods population on both IPM and conventional technology were fluctuated. Arthropods population increased in the vegetative stage and decreased in generative stage. Arthropod species numbers was higher on IPM technology field that conventional technology field. There were 118 species on IPM field and 103 species on conventional one. Total numbers of arthropods were higher on IPM field than conventional one. Equally community index between both fields was 0.009 and arthropods composition between both fields was similar. Dominancy index (Id), equitability level (E), diversity index (H`) and species richness (R) on IPM field was higher than conventional one.

Keywords:*arthropods biodiversity, IPM, Conventional technology, Paddy field*

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Impact of mass-rearing and gamma radiation on thermal tolerance of *Eldana saccharina* Walker (Lepidoptera: Pyralidae).

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Exposure of insects to gamma radiation in order to induce sterility, and mass-rearing to produce the large quantities required for the Sterile Insect Technique (SIT) are some of the many factors that may influence thermal tolerance at species and population level. The effect of mass-rearing and different radiation doses on the thermal tolerance of adult Eldana saccharina moths was investigated. Critical thermal maximum (CTmax) and critical thermal minimum (CTmin) were determined and compared for both genders of E. saccharina from five different treatments namely wild, mass-reared and three sterility dosages (150, 200 and 250Gy). Results showed that both wild and mass-reared moths had similar high temperature tolerances (CTmax: 44.3, 44.5°C respectively) and these values were significantly higher than those of moths exposed to gamma radiation (43.8, 43.7, 43.5°C respectively). However, wild moths had a significantly higher tolerance to low temperature (CTmin) than mass-reared and sterile moths (3.7, 4.4, 4.8, 4.9, 6.1°C respectively). In both CTmax and CTmin trials interaction between gender and treatment was highly significant ($F(4, 90) = 4.19$, $p = 0.004$, $F(4, 90) = 3.71$, $p = 0.008$ respectively). These results demonstrate the importance of knowledge of laboratory domestication and irradiation on thermal tolerance of E. saccharina. In addition to these data, further information on potential geographic and seasonal distribution will contribute to a better understanding of the working of SIT as an area-wide control strategy under different agro-ecological conditions.

Keywords:*thermotolerance, irradiation, sterile insect technique, sugarcane, integrated pest management.*

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Biology of exotic predator *Cryptolaemus montrouzieri* Mulsant on an invasive mealybug, *Phenacoccus solenopsis* Tinsley under laboratory conditions.

Tajwer Sultana Syed
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Studies on biology of Cryptolaemus montrouzieri Mulsant were carried out on an invasive mealybug, Phenacoccus solenopsis Tinsley under laboratory at Entomology Department, Sindh Agriculture University, Tandojam, Pakistan. Egg incubation period of C. montrouzieri recorded in present study was 4.10±0.09 days (Mean±S.D). The incubation period remained almost same during the three generations of study. There were four larval instars; the duration of different instars was 1.47±0.39, 2.44±0.41, 3.29±0.58 and 3.33±0.48 days, respectively. There was some insignificant variation in larval stages in three generations. Pupal period of C. montrouzieri varied from 5.97±0.4 to 6.97±1.04 days during different generations. Female live longer than males ,the average life span of male varied from 21.8±6.16 to 25.6±4.47 days and that of female from 27.6±4.45 to 34.3±2.94 days. The fecundity of female C. montrouzieri varied from 113 to 399 eggs and 52.9 to 86.75% eggs were fertile. The pre-oviposition period of C. montrouzieri ranged between 8.5 to 9.8 days, oviposition period from 17.3 to 21.3 days and post-oviposition period from 1.9 to 4.4 days. The sex ratio of C. montrouzieri was almost 1:1 and females copulated 38.1 to 41.8 times during their adult life with the total duration of copulation ranging from 549.5 to 567.4 minutes .The duration of life cycle of C. montrouzieri varied from 48.46±4.12 to 56.24±2.76 days during different generations.

Keywords:*Cryptolaemus montrouzieri, biology, life cycle*

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An empirical predictive model for the flight period of the ambrosia beetle *Platypus koryoensis* (Coleoptera: Platypodinae)

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From 2007 to 2009, field studies were conducted in four Quercus mongolica forests in Korea to develop an empirical predictive model for the flight period of the ambrosia beetle Platypus koryoensis. The median date of the flight period (when 50% of beetles had flown) was estimated using a cumulative Weibull function based on the cumulative proportion of all adult trap catch in the field. The pooled cumulative proportion of flight period versus degree days was fitted by the Weibull function. The relationship was most significant using 5.9 °C as the baseline temperature for development, suggesting that this temperature was the lower developmental temperature threshold of P. koryoensis. The explanatory power of the model was 89% and the model was validated by field observations in 2011 at one of the sites. The influence of climate change on the flight period of this ambrosia beetle is briefly discussed.

Keywords:*climate change, degree-days, lower developmental temperature, phenology, Weibull function*

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Estimates of pistachio yield losses caused in pistachio by pistachio psylla nymphs, *Agonoscena pistaciae* (Hemiptera: Psyllidae)

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The pistachio psylla, Agonoscena pistaciae Burckhardt and Lauterer is a major pest of pistachio trees, Pistacia vera L., through pistachio growing in Rafsanjan, Iran. The objective of this study was to determine the effect of different psylla nymph density and yield loss under field condition. Six different density levels of nymph (control, very low, low, median, high and very high density) established on pistachio trees by different insecticide dosages. The densities were monitored weekly by direct counting of nymphs per leaf from the median part of the canopy. Mean nymph density at each level was 0.6, 12.8, 32.5, 54.7, 83.8 and 114.7, nymphal-days, per leaf, respectively. After harvest, the yield loss was determined at each treatment. The yield loss was significantly affected by the number of nymphs. Mean weight of 1000 nuts in control, very low, low, median, high and very high density determined 834.4, 786.5, 741.7, 716.3, 625.6 and 477.4 g, respectively. Mean yield loss estimated 0, 47.9, 92.7, 118.1, 208.8 and 357 kg/h, respectively. Mean non-splitting determined 29, 34.2, 52.2, 59.4, 85.8 and 99.5%, respectively. Therefore, with increasing the nymph density, yield loss and percent of non-splitting increased, significantly. So, this insect is an economic pest of pistachio trees cultivar Ohadi in this area.

Keywords:*pistachio psylla, Agonoscena pistaciae, Yield loss*

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Impact of different pheromone traps installation on the capture and damage intensities of fruit fly species in various host crops

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Fruit flies are the most important limiting factor in the quality production of fruits not only of orchard crops (guava, citrus, mango, banana, cocconuts, pine apple, ber, etc) but also of field crops (cucurbits, tomato, brinjal, pepper etc.). A complex nature of fruit flies trapping on different host plants as well as species specificity to various pheromones has been observed. The present studies were carried out to explore the species distribution and their damage intensities in various host plants like cucurbits, mango, guava and citrus and well as to investigate the species specificity of two pheromones (Methyl euginol and butanone acetate) at two places during 2010 and 2011. The fruit flies trapped in both types of pheromone traps were identified on the basis morphological keys. The fruits from treated (where pheromones were installed) and untreated fields and orchards of various host crops were collected at weekly intervals, observed for infestation in the laboratory and categorized into infested and unifested to calculate % fruit infestation. The infested fruits were placed on sterilized sand in plastic jars till the larval pupation. The pupae from sand were sieved and placed petridishes inside the incubator till adult emergence. The adult emerged were then identified and classified into different species. The fruit fly species and fruit infestation data obtained at different localities, host crops and pheromone traps were statistically analysed and the results are discussed.

Keywords:*Fruit flies, species richness, infestation, host plants*

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O401M26

IPM

O401

Status of mango stone weevil, *Sternochetus mangiferae* Fab. (Curculionidae: Coleoptera) and its area-wide management leading to control and localized eradication in south India

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The stone weevil, Sternochetus mangiferae Fab. (Coleoptera: Curculionidae) is a global quarantine pest and is serious in south India. The loss caused due to premature fruit drop and spoilage of pulp amounts to 30 to 40%. Ecological and management studies from 2001 to 2012 have helped in developing area-wide management strategies which is a combination of mechanical and insecticidal interventions, combining bark junction cleaning and timely spray intervention at oviposition of weevils when fruits have a diameter of 2-4 cm (poultry egg-size). The Integrated Pest Management have shown excellent results in south India. In Dharwar district of Karnataka, weevils have been controlled 100% in orchards of Alphonso. In Vellore district of Tamil Nadu the weevil infestation has come down from 40% to <2%. In a stretch of > 200 acres in Chitoor district of Andhra Pradesh the weevil has been eradicated since 2007. This IPM intervention has caught the attention of national and international quarantine agencies. This augurs well to mitigate risk and create pest free areas. It was found that by this intervention, farmers save about 25-40% of their mangoes, resulting in about 1000 kg more yield/acre. This gives an additional income of Rs 10000 to 12000 per acre (Rs 50 = 1US $). The cost benefit ratio was worked out to 1:12 In these areas, the numbers of processing industries have gone up by about 30% in the last five years, generating increased pulp export and rural employment. The support given by NABARD and ICAR is gratefully acknowledged.

Keywords:*India, IPM, Mango, Sternochetus mangiferae, Weevil*

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IPM

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Evaluation of some botanical extracts, adult food-baits, oviposition-baits, coloured egg-receptacles and cultural practices and their possible integration for IPM of melon fruit flies.

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Studies were carried to evaluate performance of aqueous extracts of some, adult food-baits, Oviposition-baits and egg-receptacles in the laboratory by olfactometer as well in the field on bitter gourd crop from 2006 to 2009; whereas, some cultural practices like sowing times, plant-to-plant distance, sowing methods and sanitary measures were evaluated in the field of bitter gourd crop. The results revealed that ginger, garlic and neem extracts (botanicals), GF-120 (adult Food-bait), yellow, white and orange colored Oviposition bait and egg-receptacles with water melon juice plus paste, Hang Sowing Method (HSM) and early sowing, with a plant to plant distance of 45 cm, performed better causing significant reduction in fruit fly infestation as well as yield losses/plant and significant increase in the number of marketable fruit/plants and marketable yield/plant. The most effective melon fruit flies management tactics, screened, through different experimentations, were integrated into different IPM-Models, which were evaluated under field condition. The result revealed that IPM-MODEL-2, IPM-MODEL-3, IPM-MODEL-4, IPM-MODEL-5, IPM-MODEL-6 and IPM-MODEL-7 were effective and economical resulting in marketable yield 6-7 times higher (CBR=1:6.2 to 1:7) than control (IPM-Model-8) and 2-3 times higher than conventional IPM-Model-1 (CBR=1:4.5). In conclusion, implementation of any of these IPM- Models in bitter gourd cropping system would reduce the infestation of melon fruit fly infestation and ensure the significant increase in bitter gourd yield with effective and economical cost-benefit ratio (CBR).

Keywords:*Botanicals, Mechanical control, Cultural control, IPM, Fruit Fly*

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Alternative host plants of *Megalurothrips sjostedti* and *Frankliniella occidentalis* in French bean agroecosystems in Keyna

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Sound management of agricultural pests requires knowledge of the pest, both on the target crop and the surrounding plant community. At the onset of flowering of French beans, there is rapid population build up of Megalurothrips sjostedti Trybom and Frankliniella occidentalis Pergande in the flowers. The extent to which co-existing thrips species utilize common resources is unknown in Kenya. Understanding the role of alternative hosts on population dynamics of thrips in French beans may lead to development of novel management strategies. Collections of alternative host plants were made at jkuat, kaguru and machakos Farmers'Training Centres. Presence of larvae and adults on various potential host plants were monitored by direct observation of flowers of wild and cultivated crops, up to 0.2km of the French bean fields. Flower samples were taken to the laboratory where adult thrips and larvae were extracted by dissecting the flowers. Adult thrips were identified under a binocular microscope. Larvae were removed and put in emergence cages, supplied with flowers of the plants from which they were extracted, and reared at room temperature until eclosion to adults. Many alternative hosts were found in flower after the rains, but also throughout the year. There were plants in flower at any given time of the year. Thrips, therefore, never lacked alternative hosts. Frankliniella occidentalis was found in more plant families relative

Keywords:*Bean Flower Thrips, Western Flower Thrips, Polyphagy*

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Biology and influential abiotic environmental variables for the beet leafhopper (*Circulifer tenellus*) in the Columbia Basin of Oregon and Washington

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The beet leafhopper, Circulifer tenellus Baker, is responsible for transmitting a phytoplasma that causes purple top disease in potatoes in the Columbia Basin of Oregon and Washington. This phytoplasma is referred to as beet leafhopper-transmitted virescence agent (BLTVA). Beet leafhoppers are a significant pest in many areas around the world, including much of Eurasia. The following study was designed to predict the population dynamics of the beet leafhopper in the Columbia Basin based on abiotic environmental factors. From 2006 through 2008, individual sticky card traps were placed at approximately 100 sites in Oregon and Washington. Beet leafhoppers collected by these traps were evaluated weekly. According to the 2006 through 2008 survey data, beet leafhopper populations typically peak in early June; a second peak occurs early to mid-October. Populations fluctuate significantly between years, and the abiotic environmental factors were analyzed to explain these extreme fluctuations. The correlations between weather, location and beet leafhopper populations will be discussed, as well as incorporated with beet leafhopper biology. Due to the inclusion of abiotic environmental factors, these results may provide useful information for other geographic locations.

Keywords:*phytoplasma, beet leafhopper transmitted-virescence agent, potatoes, purple top disease, population*

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O401M30

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O401

The correlation between artificial night lightings and attraction response of insect

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The artificial night lighting gives the convenience of human life and it enables human nighttime activity. However, strong lamp causes the 'light pollution' because some are installed without special regulation and planning. The insects are specially attracted well by the lamp and cannot escape from the light and eventually killed by natural enemies or human's activities, naturally. It causes a decrease of population and it is very critical damage for the ecosystem because the insect is very important primary or secondary consumer on the food chain. This research will provide the foundational data and ground about the ecological effect by the light pollution. We try to find the attraction feature of insect that has high diversity compare with other groups, and predict the degree of disturbance to the ecosystem. We designed three main experiments. The experiments are about the attraction response of diverse artificial lightings have different brightness and colors, and the others are used a lot in human's life. We utilized light trap as attracting insect and counted the number of occupied insect around the each lamp. We measured the illumination and UV-A of all lamps, and tried to find the correlation. As a result, the lightings that attract much more number of insects have higher illumination and UV-A than the others, but there was no relationship with color. Also, the lightings have high UV-A and low illumination attract much more number of insects than low UV-A and high illumination.

Keywords:*light pollution, artificial night lighting, population decreasing, attraction response, surrounding ecosystem*

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A decade with *Solenopsis invicta* (Hymenoptera: Formicidae): Taiwan's experiences on fire ant control

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Since its accidental invasion, red imported fire ant (Solenopsis invicta) was first observed in Taiwan in 2003 and until 2012, more than 50,000 ha land is already been infested. To prevent S. invicta from further spreads and subsequent damages, National Red Imported Fire Ant Control Center (NRIFACC) was established in 2004. Baits with either toxicants or insect growth regulators are the main control agents in Taiwan against S. invicta. To ensure the quality of fire ant treatments, GIS mapping of the delivery and tracking of the treatments were adopted. Similarly, sticky papers and pitfall traps are randomly placed to monitor the dose of fire ant baits applied. The potato chip based bait traps are used to confirm the presence or absence of fire ants at treated or suspected areas. The NRIFACC is assisting governmental agencies to formulate and execute the regulations to restrict the human-caused fire ant disseminations. NRIFACC organizes regular trainings/workshops to train local resource persons and use other mass media to create public awareness towards the fire ant management. Along with fire ant control efforts, numbers of scientific studies including development of water tolerant fire ant bait, exploration of native competitor ant species, nature based control materials, and population genetics are on the progress to facilitate the national fire ant control program. Recently developed new carrier based fire ant bait (T-bait) that tolerates water or high humidity could be a potential control tool for the Taiwan, China, Hong Kong and other areas.

Keywords:*Red imported fire ant, eradication program, fire ant bait, monitoring*

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Biological parameters and feeding efficiency of *Chrysoperla carnea* on different hosts

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Study on the biological parameters and feeding efficiency of Chrysoperla carnea on different hosts was carried out in the laboratory of Plant Protection, S A U, Tando Jam. The results indicated that the pre-oviposition period was 7.8±1.17, 7.98±1.16, 7.4±1.39 and 6.9±1.51days on S. Cerealella, E. insulana, H. armigera and P. gossypiella respectively. Post-oviposition period of C. carnea lasted for 3.1±1.26, 5.3±2.63, 6.3±1.42, 8.1±1.36 and 4.3±1.15 days on S. cerealella, E. insulana, H. armigera and P. gossypiella, respectively. The mean duration of the first, second and third larval instars of C. carnea on different hosts was 3.1±0.056, 3±0.66 and 3.5±0.84 on S. cerealella, 3.25±0.58, 3.3±0.78, 4.15±0.63 on E. insulana, 3.89±1.12, 4.3±0.63, 3.5±0.45 on H. armigera and 3.1±0.68 on P. gossypiella 3.67± 0.65, 2.95± 0.45, 3.1± 0.68. The males lived for mean duration of 14.6±4.07, 35.8±1.78, 30.31±1.87 and 33.37±1.83, days on S. cerealella, E. insulana, H. armigera and P. gossypiella, respectively. The mean feeding percent per day of 1st, 2nd and 3rd instar larvae of C. carnea was 28.5, 56 and 87.5 percent eggs of S. cerealella, 29.25, 62.25 and 89.5 eggs of E. insulana, 24.25, 63 and 86.25 eggs of H. armigera and 29.25, 59 and 90.5 eggs of P. gossypiella. It also observed that the third instar was most voracious feeder. From data it was concluded that the predator can be reared on all the five hosts. Therefore, S. cerealella eggs are the ideal food for rearing the predator because of its availability round the year.

Keywords:*Bioloy, Efficiency, Chrysoperla carnea and hosts*

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Morphometrics and Integrated control of *Macrotermes bellicosus* (Isoptera: Termitidae) on newly established cocoa plantation in Ibadan, Nigeria

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An unprecedented outbreak of termite infestation was observed at the Cocoa Research Institute of Nigeria Headquarters (CRIN), Ibadan during the 2005/06 cocoa seasons in which several newly established and old plantations were put under threat. An integrated pest management (IPM) approach which comprised physical, cultural and chemical methods was put in place to control the infestation and damage caused by this pest in order to provide a healthy environment for the growth of the newly established cocoa plants in the field. In a two hectare plantation consisting of about two thousand and eighty (2, 080) young cocoa plants, every stand showed symptoms of attack by termites while a total of 176 termite mounds were counted. The characteristics and morphology of mounds as well as the different castes of termites were described and reported. The average circumference of mound measured was 6.1 meters with a range of 3.6 to 8.6 meters while the mean height was 1.59 meters with a range of 0.7 to 2.8 meters. The most numerous and smallest in size of the castes were the workers with a body length of 1.24cm and body width of 0.4cm. Both physical and chemical treatments were carried out on all mounds while dry and decomposing logs, stems and fallen branches were removed from the plantation for use as firewood. One year after treatment, only 415 (20%) young cocoa stands which could not survive previous season attacks, were replanted. The plantation was subsequently monitored for two seasons for termite infestation.

Keywords:*Integrated Pest Management, Macrotermes bellicosus, Mophometrics, Caste description*

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O401M34

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O401

Distribution of potato leafminer fly, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) and its damage in potato crops in Nepal

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Potato leafminer fly, Liriomyza huidobrensis (Diptera: Agromyzidae), is an economically important pest of potato and ornamental plants in Nepal. Though pesticide application is common control practice against this pest, pesticide resistant of this pest stalls proper management. Distribution and damage assessment were conducted in major potato growing districts (Kavre, Lalitpur, Kathmandu, Bhaktapur, Makwanpur and Sindhupalchowk) of Nepal during 2009 and 2010. A total of 199 farmers fields were surveyed from the six districts. Also, 10 fields were assessed for damage in each district by randomly selecting 10 potato plants from each field. To quantify level of damage, plant samples were taken to laboratory. Damage level was compared according to location, altitude, variety, and cropping systems. Distribution of the pest was found mostly concentrated on the mid-hill region with high level of infestation. The damage level varied among the districts. Highest damage was recorded in Lalitpur (1332msl) district and least was in Sindhupalchowk district (2301msl) in both years. No parasitism was recorded in high altitude (2301msl). 'Cardinal' and 'Lal-gulab' potato varieties showed highest infestation (45%) while 'Rosita' showed the lowest (4.25%). Among cropping systems, in maize-potato-vegetable system showed higher damage (46.15%) followed by rice-potato-potato system and the root crop-fallow-potato system showed least.

Keywords:*Damage level, Liriomyza huidobrensis, altitude, varieties, cropping pattern, parasitism*

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O401M35

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Area wide implementation of integrated pest management in India for cauliflower cultivated in rainy season

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Cauliflower occupies fifth position in total production of 133.74 million tonnes of vegetables in India. Due to genetic advancement and improvement in production system, cauliflower can be cultivated throughout the years which were earlier confined to winter season only. It has resulted in quantum jump in earnings of farmers, but also has attracted a large variety of pests that are potential threat to its cultivation. In order to sustain its production farmers resort to 10-12 applications of pesticides over a period of four months to prevent the damage due to the potential pests such as Spodoptera litura, Helulla undalis, Pythium sp and Alternaria brassicae and A. brassicola in the rainy season from month of June to November. To address the problem of over use of pesticide and restore sustainable cultivation of cauliflower, integrated pest management technology comprising soil solarisation, raised bed method of developing nursery, application of Trichoderma harzianum to soil through FYM, pest monitoring through sex pheromone trapping, scouting of pest damage, collection of egg masses of S. litura, plucking of pests infested leaves, need based application of reduced risk pesticides and monitoring of insecticide resistance in S.litura was developed and validated and popularize in farmers participatory mode for state of Haryana and Rajasthan of India. The implementation of IPM technology envisaged higher economic returns, lower pest incidence and cost of plant protection and higher natural enemies' population. It can also be implemented in other states of India having similar pattern of cauliflower cultivation with similar pest problems.

Keywords:*cauliflower, IPM, S.litura, rainy season*

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O401M36

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O401

Use of *Tithonia diversifolia* and *Metarhizium anisopliae* in a lure-and-kill approach for thrips management

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The attraction of the thrips Frankliniella occidentalis to commercial thrips kairomone LUREM-TR and natural lure from flower heads of Tithonia diversifolia was studied in the field. Blue sticky trap, preferred thrips visual cue, was also included in the study. Blue sticky traps baited with flower heads of T. diversifolia captured three-fold more F. occidentalis than blue sticky traps (control). However, traps baited with kairomone LUREM-TR attracted significantly more thrips than traps baited with T. diversifolia flower heads and the control. There were no significant differences in thrips captures between traps baited with T. diversifolia and the control due to the neighbouring effects of LUREM-TR. We also investigated the possibility of integrating M. anisopliae with lures in autodissemination approach. In laboratory assays, organic extracts of the flower heads of T. diversifolia were compatible with the fungus than water extracts while LUREM-TR completely inhibited conidia of the M. anisopliae. Conidial acquisition and mortality of thrips attracted to the autoinoculation device baited either with flower heads of T. diversifolia or LUREM-TR were not significantly different between the treatments sin greenhouse experiments. The potential of using T. diversifoliaflower heads as an alternative kairomone source for a lure and kill strategy for thrips management is discussed.

Keywords:*Thrips, Tithonia, Blue, autoinoculative device*

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O401M37

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The effect of certain watermelon varieties on the biology of *Aphis gossypii* Glover (Hemiptera: Aphididae)

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The development, reproduction, and population growth parameters of the melon aphid, Aphis gossypii (Glover), on four watermelon varieties were evaluated in climatic room and growth chambers. The watermelon varieties were Crimson Sweet, Crimson Tide F1, Galactica, and Surme. Significant differences in the biology of Aphis gossypii were observed. The highest fecundity was recorded on Galactica (58.97 offspring/female), followed by Crimson Tide F1 (57.80 offspring/female), Surme (57.25 offspring/female) and Crimson Sweet (57.03 offspring/female). The highest net reproductive rate was observed on Crimson Tide F1 (22.40 offspring), while the lowest was on Surme (18.61 offspring).

Keywords:*Aphis gossypii, Biological parameters, Watermelon, Varieties*

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O402TH01

IPM

O402

Evaluating alternatives to conventional pest control in Benin

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Efficacy of Neem oil (Azadirachta indica) alone and, depending on the pest, combining with the entomopathogenic fungus Beauveria bassiana formulation Bb11, both threshold applied for controlling cotton pests, was compared to conventional calendar-based spraying with synthetic pesticides and the "Lutte Etagee Ciblee (LEC) strategy, consisting of calendar-based spraying (half dose) followed by threshold application of synthetic pesticides. The experiment was carried out with farmers in three cotton agro-ecological zones differing in rainfall, pest prevalence, and farmer practices. The neem oil, and neem oil-Bb11 treatments required 2 to 6 treatments, while conventional and LEC received 6 to 8 treatments. Percentage damaged bolls in plots receiving the neem oil and neem oil-Bb11 treatment was higher than in those having the conventional and LEC treatments, resulting in yields being 25 and 39% lower, respectively, while in one agro-ecological zone with low rainfall there was no difference. Yields in the biopesticide plots were 26-42% higher than in plots without having received pesticides, while in the conventional and LEC plots they were 44-59% higher. Natural enemy incidence was highest in the control without using pesticides and in the biopesticide plots. Overall, the LEC regime scored best, both in yield and profitability but the specific pesticides required are not made available on the market. Using the entomopathogen Bb11 and neem oil avoid many problems associated with synthetic pesticide use, but their efficacy need to be enhanced by improved formulation or combining it with other pesticides in order to become a viable alternative.

Keywords:*biopesticides, botanicals, integrated pest management, farmer participatory research*

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O402TH02

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O402

Effects of the essential oils of *Lavandula angustifolia* Mill and *Zataria multiflora* Boiss on reproduction and F1 population of *Callosobruchus maculatus* (F.)

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Inhibitory effects of L. angustifolia and Z. multiflora essential oils were evaluated on reproduction and F1 progeny production of C. maculatus. Insects culture and bioassays were conducted at 30 ±1ºC, 60±5% RH and dark condition. Essential oils were taken by hydrodistilation. The results indicated that there was a negative and significant linear relationship between reproduction of beetles and oil concentration. Similar relationships was found between F1 progeny and oil concentration. Comparison of regression slopes showed that L. angustifolia oil was more effective than Z. multiflora oil on reproduction and F1 progeny population. The essential oils analysis by GC-MS showed that the main compounds of L. angustifolia oil were linalool, 1,8-cineol, rosefuran epoxide, menthone, isomenthol and dihydro carvone (trans) and those of Z. multiflora oil were thymol, linalool and p-cymene. The results indicated that these two essential oils especially L. angustifolia oil might be suitable alternatives for stored products protection against C. maculatus.

Keywords:*Essential oils, F1 progeny reduction L. angustifolia, reproduction inhibitory, Z. multiflora.*

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Impact of *Lantana camara* leaf extracts on development and reproductive bioactivities of *Dysdercus koenigii*

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Development and reproductive bioactivities of Dysdercus koenigii as modified by the leaf extracts of Lantana camara were evaluated. Fifth instar nymphs of Dysdercus were treated with four concentrations of ethanol extract and hexane extract prepared from leaves of Lantana camara. Dysdercus exhibited significant alteration in their survival, longevity, development and reproduction when treated with ethanol extract. A significantly higher mortality was observed in the nymphs and adults when treated with higher dose of the extract. At lower concentration, the ethanol extract of Lantana influenced development and reproduction in Dysdercus. Hence the treated insects exhibited delayed moulting, supranumery instars and abnormal moulting. The adults emerged from the treated fifth instar nymph were characterized by wings deformities, malformed proboscis and shrunken body. The developmental aberrations were dose related and also the effects were more pronounced when the extract was applied to newly emerged fifth instar. The ethanol extract of Lantana also affect the reproductive behavior, fecundity and fertility of Dysdercus. This resulted in reduction in the number of egg batches, number of eggs in each egg batch and total number of eggs laid by the female in its entire life span. More than 50% of eggs laid by these females were sterile. The hexane extract of Lantana leaves had more pronounced effect on the mating behavior of Dysdercus. Consequently, treated males showed lesser number of mounting attempts and delayed mating and decreased percent successful mating. Possible role of JH mimicking moleculles present in Lantana in development and reproduction of Dysdercus was discussed.

Keywords:*Dysdercus, Lantana, Development, Reproduction*

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Green synthesis of silver nanoparticles (AgNps) and azadirachtin for the control of Asian palm weevil *Rhynchophorus ferrugineus*. (Olivier) (Coleoptera: Curculionidae)

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Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biological applications of Neem tree, Azadirachta indica, A. Juss. (Meliaceae), has been widely studied due to the presence of a great number of compounds with insecticidal properties and that are effective on several pest species. The invasive red palm weevil, R. ferrugineus (Olivier) (Coleoptera: Curculionidae), has become the major pest of palms trees in India. Economically speaking of the 40 millions coconut palms was affected by the pest which accounts to 200,000 palms every year in India. In the present investigation under the laboratory condition R. ferrugineus larvae, pupae and adult was treated with azadirachtin and synthesized AgNps . After 48 hrs, Larval, pupal and adult mortality was determined. AgNps shows highest larval mortality (99.3%), pupicidal (78.3 %) and adulticidal (69%) activity against R. ferrugineus in all the tested concentrations (3, 6 and 10 ppm) than the azadirachtin treatment. Therefore, the present investigation clearly exhibit that AgNps could serve as a potential of highest mortality rate against the R. ferrugineus larvae, pupae and adult in laboratory conditions.

Keywords:*Silver nanoparticles (AgNps), Azadirachtin, Rhynchophorus ferrugineus*

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Screening of some citrus oils against two strains of Khapra beetle (*Trogoderma granarium*) Everts (Coleoptera: Dermestidae) collected from Vehari and Faisalabad districts of Punjab, Pakistan

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Mansoor ul Hasan, Muhammad Sagheer, Qurban Ali, Imran Faraz Department of Agricultural Entomology, University of Agriculture Faisalabad, Pakistan E.mail: mansoorsahi2000@yahoo.com In the present study, an attempt was made to examine the effects of essential oils of four citrus species (Citrus paradisi, Citrus sinensis, Citrus aurantium and Citrus reticulata) on two different strains (Faisalabad and Vehari) of T. granarium. Grubs of T. granarium were exposed for 3, 6 and 9 days to grains treated with 2, 4 and 8% concentrations of citrus peel essential oil under laboratory conditions. At concentration of 8% C. aurantium essential oils were highly effective against T. granarium than other citrus species with respect to percent mortality (27.30%), followed by C. reticulate (26.65%), C. sinensis (22.36%) and C. paradisi (20.00%). From data it has been observed that Faisalabad strain is susceptible than Vehari strain against T. granarium. At 8% concentration F1 progeny failed completely to develop in case of C. sinensis. Data regarding grain loss caused by T. granarium showes that minimum grain loss occurred in case of C. aurantium treated grains, followed by C. paradisi, C. sinensis and C. reticulate. It could be concluded that citrus essential oils possess excellent insecticidal, growth inhibition and antifeedant activity against T. granarium.

Keywords:*Citrus species, essential oils, mortality, grain loss, T. granarium*

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Bio-efficacy of botanicals and bio-pesticides in the management of defoliator complex of *Gloriosa superba* (Linnaeus)

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Gloriosa superba is a medicinal plant of immense value in the traditional systems of Indian medicine. Seeds contain colchicines and thiocolchicocide and possess many medicinal and curative properties. G. superba is devasted by defoliators like Polytela gloriosae, Plusia signata and Spodoptera litura. Field experiments were carried out at the Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India and in the farmers' holdings to study the field efficacy of bio-pesticides and botanicals for the management of above defoliators infesting G. superba. Results of the experiments revealed that among the treatments, flavonoids at one ml per litre of water (Max Ranger-D2®) recorded superiority in the management of defoliators starting from third day after foliar spray and found to be statistically on par with standard chemical check, quinalphos at 2 ml per litre. Pungamia pinnata seed oil 3 per cent was next in the order of efficacy, followed by azadirachtin 1 per cent and neem seed kernel extract 5 per cent. Efficacy of Bacillus thuringiensis @ 250 larval equivalents was realized only at seven days after treatment and persisted even after 14 days of second spray. At 14 days after treatment, Bt was second in the order of efficacy next to chemical pesticides and flavonoids. From the results of both the field trials, flavonoids are adjudged and recommended as the best alternative to chemical pesticides in gloriosa ecosystem and can be recommended as one of the components in organic pest management.

Keywords:*Gloriosa superba, Polytela gloriosae, Plusia signata, Spodoptera litura, Flavonoids*

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Respiratory toxicity of *Thuja orientalis* on different life stages of *Tribolium castaneum*

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Tribolium castaneum is one of the most important storage pests which cause significant losses on stored products. Utilization of chemicals has unfavorable effects on flour and makes it unusable. Therefore it is necessary to substitute the conventional chemicals with appropriate alternatives. Since plant extracts do not have uncomplimentary effects, hence seem to be appropriate alternatives for chemicals. In this research, the respiratory toxicity of Thuja orientalis extracts has been evaluated against eggs, larvae and adults of Tribolium castaneum. In this regard, essence of Thuja orientalis fresh leaves have been extracted with clevenger apparatus and hydro distillation method, and its toxicity have been tested on 1-7 days old adults, 14-16 days old larvae and 1-3 days old eggs of Tribolium castaneum in 25±1°C temperature and 70±5% relative humidity and dark conditions with 10-83 µl/350 ml air with 4 repetition. Experiments revealed that the mortality rate considerably increases in higher extract's concentrations and exposure times. Additionally results showed different life stages of Tribolium castaneum had diverse susceptibilities to the extract as more susceptibility of the adults compared to that of eggs and larvae. The calculated LC50 of this study in 24 hours exposure time was 18.81± 2.15 µl/350 ml airs for adults, 29.48± 1.44 µl/350 ml airs for larvae and 40.02± 1.48 µl/350 ml airs for eggs. These Results indicated that the extracts of Thuja orientalis could be introduced as a suitable alternative to chemicals.

Keywords:*Tribolium castaneum, Thuja orientalis, LC50.*

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Evaluation of *Solenostemma argel* extracts for their potential larvicidal activities against *Anopheles arabiensis* Patton (Diptera: Culicidae)

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Abstract: Among the prevalent mosquito species in Africa Anopheles arabiensis is the important malaria vector distributed in most countries. The main control tactics adopted depend largely on the application of synthetic chemicals. However, the socio-economic and environmental drawbacks of these chemicals are well known in everywhere. Therefore, this work was proposed to study the occurrence of A. arabiensis in central semi arid region of Sudan, and to evaluate different extracts of Solenostemma argel on its control. Leaves and seeds of the plant were extracted using water and organic solvents (viz., petroleum ether and ethanol), and their phytochemical constituents were analyzed. Bioassays of such extracts were conducted against the 4th instar larvae of the mosquito, where the mortality and residual effects were compared. The results revealed that the relative occurrence of A. arabiensis were higher around the River Nile. The chemical constituents of the tested extracts showed variable levels of secondary metabolites, i.e., alkaloids, saponins, flavones, flavonoids, amino acids, triterpenoids and sterols. The highest mortality percent (90%) was achieved by the seeds petroleum ether extract (LD50 1000ppm), which was comparable with that of the standard insecticides (Malathion and Abate) used. This extract also gave the best residual activity after one month. However, the mortality means of different botanical treatments increased with increasing concentrations and time of exposure. In conclusion, the findings proved the potent insecticidal activity of S. argel against A. arabiensis, and suggested more studies to be utilized commercially as natural mosquito larvicide.

Keywords:*Solenostemma argel, Anopheles arabiensis, larvicide, mortality, residual effect.*

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Comparative assessment of insecticidal effect of some wood treated with plant extract on termite

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This study compares the insecticidal effect of some plant extracts on some wood species against termite attack. Five different wood species were used for the study (Gmelina arborea, Sterculia rhinopetala, Cordia millenii, Cola gigantea, and Ceiba petandra. Neem seed, Liquefied biomass and undiluted macerated Neem leaves were used to compare the most effective plant material on the wood species on field test. The wood samples were oven dried, cold soaked for 24hours and conditioned for 48 hours and randomly placed in termiterium. Evaluations of termite attack on wood samples were carried out bi-weekly for twelve weeks. Ceiba petandra (89.43%) treated with Liquefied biomass recorded the highest mean percentage absorption, followed by C. petandra treated by neem seed (64.09%) and one treated by neem leaves (56.36%) had the least absorption. Ceiba petandra treated wood had the highest value (2663.93 g/cm3) for retention followed by wood treated with liquefied biomass, while wood species treated with neem seed extract had (6.45 g/cm3) and those of neem leaves (178.65 g/cm3). The least percentage weight loss was recorded in Gmelina arborea, liquefied biomass treated wood had (87.43%) followed by neem seed treated wood (39.21%) while the highest weight loss was recorded in neem leaves (54.80%) treated wood. There was little attack on the treated wood species but all the untreated samples were destroyed. Termite attack on wood treated with liquefied biomass was significantly lower compared with other wood species treated with other plant materials.

Keywords:*Liquefied biomass, Neem seed, Neem leaves, Hard wood, Termite*

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Toxicological and repellent impact of essential oils of *Citrulus colocynthus, Citrus pardesi, Solanum nigrum* and *Cuminum cymintion* against *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae)

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Toxicological and repellent effects of essential oils obtained from Citrulus colocynthus, Citrus pardesi, Solanum nigrum and Cuminum cymintion were evaluated, under laboratory conditions, against Trogoderma granarium (Everts), a serious pests of stored cereals. Essential oils were extracted using acetone solvent in Soxhlet's apparatus. Three concentrations viz., 5, 10 and 15% of each plant extract were used for both bioassays. Data of percent morality was observed after three different post treatment intervals viz., 3, 5 and 7 days. The results revealed that all the treatments differed significantly from one another at all the concentration levels of the plant extracts and at all the post treatment intervals. The application of oil extract of C. Colocynthus showed maximum mortality of the pest at all the concentration levels followed by Solanum nigrum, Citrus pardesi and Cuminum cymintion. The exposure period of 7 days showed maximum mortality of the pest i.e. 23.75% in C. colocynthus followed by 14.17, 12.92 and 12.08 in oil extract of S. nigrum, Citrus pardesi and Cuminum cymintion, respectively. From the results it was found that C. colocynthus at maximum concentration and longer exposure period found to be the most effective as compared to other oil extracts of botanical origins. Experiments were also conducted to evaluate the repellent effects of these plant extracts. Results reveals that highest repellency (82.66%) was observed against C. Colocynthus followed by C. pardesi (76.33%), C. cymintum (68.36%) and S. nigrum (62.66%).

Keywords:*Khapra beetle, plant extracts, repellence, mortality*

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O403TU01

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O403

Deep mining of transcriptome data of four Lepidoptera pests

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Chilo suppressalis, Scirpophaga incertulas, Cnaphalocrocis medinalis and Spodoptera exigua are important Lepidoptera pests, which cause great reduction in rice production. However, the gene data of these pests are scarce. We sequenced the transcriptome of four Lepidoptera pests using Illumina Solexa technique. The qualified reads were assembled into contigs by the software Trinity. In total, there are 69977, 64069, 73284, 31414 contigs, of which 27,347, 28,582, 36,802 and 18,592 contigs were annotated as protein-coding transcripts for C. suppressalis, S. incertulas, C. medinalis and S. exigua, separately. The similarities of nucleotide and protein sequences between abovementioned pests and Bombyx mori were analyzed. The results indicated that the C. suppressalis and S. incertulas are the most close species among the five insects. Though S. incertulas is monophagous whereas C. suppressalis is ploryphagous, S. incertulas has the more digestive enzyme transcripts than C. suppressalis. The reason is worthy of further investigations. It is possible that there are some symbiotic bacterias which enabled C. suppressalis to digest more plants. We also found some insecticide resistance genes including acetylcholine receptor, acetylcholinesterase, glutathione S-transferase, p450, and ryanodine receptors in each transcriptome data, which can be used to further analysis.

Keywords:*Transcriptome, Lepidoptera, pests, bioinformatics analysis*

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O403TU02

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Resistance characteristics of different *Brassica* species to mustard aphid, *Lipaphis erysimi* Kalt. (Hom.: Aphididae)

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The mustard aphid, Lipaphis erysimi is one of the most severe pests of canola in Iran and other tropical regions in the world. Three species of Brassica including B. napus (cv. Licord, Elite, Okapi, Hyola 401, and RGS003), B. juncea (cv. Land race) and B. rapa (cv. Park land) were screened for antixenotic and antibiotic resistance to L. erysimi under greenhouse condition at 25±2℃, 60±5% r.h. and a photoperiod of 16:8 h. (L: D). The results of antixenosis test indicated that density of L. erysimi was significantly different on canola cultivars at different hour counting ($P < 0.05$ and $P < 0.01$ after 48 and 72 h, respectively). Aphid density on Elite, Okapi, and RGS003 cultivars was significantly higher than that on other cultivars. In antibiosis test, significant differences were detected among canola cultivars in aphid life-table parameters including intrinsic rate of increase (rm) ($P < 0.05$), net reproductive rate (Ro) ($P < 0.05$), mean generation time (T) ($P< 0.05$) and finite rate of increase (λ) ($P < 0.05$) with the exception of population doubling time (DT) ($P > 0.05$). The aphids had the highest rm on Land race cultivar (0.324 female/female/generation) and lowest on Elite (0.278 female/female/generation). Our results suggested the cultivars Elite, Okapi and RGS003 as the least susceptible host for L. erysimi. The resistant canola cultivars identified in the present study could be used for the management control of mustard aphid.

Keywords:*Canola, Lipaphis erysimi, Iran, Resistance*

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Adaptation of the brown planthopper to a highly resistant rice variety PTB33

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The Indian rice variety PTB33 is one of the most resistant to the brown planthopper (BPH), Nilaparvata lugens (Stal), and has been used extensively in breeding programs aimed at increasing rice resistance to biotic stresses. The sources of resistance in PTB33 are still not fully understood, but it appears that the variety may contain several resistance genes (bph2, Bph3, Bph17), thus contributing to its durability. We examined the resistance of PTB33 against six BPH populations from the Philippines. Planthopper weight-gain, longevity and egg-laying were lower on PTB33 than on the susceptible standard TN1. We artificially selected for virulence in all six populations by rearing planthoppers on the resistant variety over 20 generations. Adapted planthoppers had increased survival, longevity and reproductive output when compared to non-adapted colonies. We then screened, using the adapted and non-adapted BPH colonies, 30 differential rice lines with different BPH genes for their effects on planthoppers. The results were used to determine likely or analogous BPH resistance genes present in PTB33. We also monitored sequentially the abundance of endosymbionts in the pre-adapted and adapted colonies to examine possible relations between endosymbionts and planthopper virulence.

Keywords:*brown planthopper, Nilaparvata lugens, PTB33, adaptation, rice, endosymbiont*

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RNA interference in the migraltory locust

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Locusts are one of the most destructive agricultural pests in the world, causing considerable economic and ecological damage. They are also an important physiological model, as they exhibit responses to environment that manifest themselves as phenotypic changes in behavior, color, metabolism, development, and morphology, known as locust phase polyphenism. We observed that the locust has pronounced sensitive systemic RNAi in response to dsRNA injection for both broadly-expressed as well as tissue-specific genes but has no senstive response to dsRNA feeding. The evidence from in vivo loss-of-function and in vitro experiments suggested that the LmSID-1 gene was not required for systemic dsRNA uptake and spread, implying the existence of alternative mechanisms underlying insect systemic RNAi, which may be different from Caenorhabditis elegans or mammals.

Keywords:*Locusta migratoria, dsRNA, SID-1, pest control, gene expression*

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Screening of lethal genes for feeding RNAi by leaf disc-mediated systematic delivery in *Tetranychus urticae*

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The screening of effective lethal genes was conducted via the systemic delivery of dsRNA for the RNAi-based management of Tetranychus urticae. Four candidate genes (coatomer protein complex, T-COP; M1 metalloprotease, T-M1MP; Ribosomal protein S4, T-RPS4; A subunit of V-ATPase, T-VATPase) with a reference genes (EGFP) were tested for the experiment. The permeated dsRNA to the leaf disc (ca. 30 mm diameter) was detected at 12 h after treatment, indicating that dsRNA could move through veins on the leaf. In the evaluation of mortality, the T-COPB, T-RPS4 and T-VATPase exerted higher toxicities at 72 hour after treatment among four dsRNA tested. Gene knockdown corresponding four dsRNA were confirmed by qPCR, suggesting that the dsRNA could suppress the target mRNA through feeding dsRNA. In summary, the dsRNA delivery via leaf disc was effective in screening lethal genes and some genes, such as T-COP, T-M1MP, T-RPS4 and T-VATPase, can be applicable for establishing a RNAi-based control system against T. urticae.

Keywords:*RNAi, dsRNA, Systemic delivery, Tetranychus urticae*

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Insect resistance to Bt plants: Time to start paying attention?

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Bt plants provide a valuable and often environmentally friendly tool for managing insect pests. Growers of non-Bt corn are currently benefiting from area-wide suppression of insect pests by Bt corn, while organic corn growers use Bt sprays to control the same pests. Failure of Bt corn to control target pests is likely to encourage the use of less environmentally benign chemical insecticides, thus increasing health risks to the general population. Clearly, the preservation of this technology is important for people far beyond the producers of Bt corn. Unfortunately, recent field failures of Bt corn due to the damage caused by resistant rootworms confirm that it is potentially vulnerable to insecticide resistance. Yet, current resistant management plans are based on several somewhat questionable assumptions, which should be further scrutinized by the scientific community.

Keywords:*Bt, GMO, insecticide resistance*

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Sources of resistance in wheat to sunn pest (*Eurygaster integriceps* Puton) identified using the focused identification of germplasm strategy (FIGS)

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Sunn pest, Eurygaster integriceps Puton, is the most important insect pest of wheat in West and Central Asia. Host plant resistance is the most economical and practical means for controlling insects. The Focused Identification of Germplasm Strategy (FIGS) enables large genebank collections to be "mined" for specific traits such as insect pest resistance. FIGS is based on the paradigm of co-evolution of, and interaction between, host and pest. It analyzes the agro-climatic characteristics of sites from which plant germplasm was originally collected, to make predictions of best-bet accessions that are most likely to contain the trait of interest. FIGS was used to identify trait-specific best-bet subsets from a total of 16,000 geo-referenced accessions conserved in the genebanks of the International Center for Agricultural Research in the Dry Areas, the Vavilov Institute and the Australian Winter Cereals Collection. The selected material was field screened in cages under artificial infestation in Syria for resistance to Sunn pest at the vegetative stage. Out of a total of 1000 wheat accessions tested, 10 durum wheat and nine bread wheat lines showed a very good level of resistance at the vegetative stage. The results also showed the effectiveness of the FIGS approach in identifying genotypes with valuable traits. FIGS has the potential to reduce the resources required to exploit genetic resource collections. Crosses with the identified sources of resistance have been made to develop resistant germplasm to Sunn pest and also mapping populations to identify chromosomal region(s) and molecular markers linked to the resistance.

Keywords:*Wheat, Eurygaster integriceps, resistance, FIGS*

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O403TU08

IPM

O403

The shift towards controlling secondary pests on Bt-cotton and the implication thereof for Integrated pest management on Bt-cotton.

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With the introduction of new technologies on cotton in South Africa, such as single gene traits and double gene traits, which express not only insect tolerance but also herbicde resistance, the emphasis of chemical control on Bt-cotton varieties has shifted towards controlling the secondary pest complex on cotton. The bollworm complex is controlled by the inherent characteristics of the cotton varieties, i.e. the Bt-endotoxin, which is expressed in the plants, and bollworm numbers are kept under economically damaging levels throughout the season. Fewer chemicals aimed at bollworm control are subsequently sprayed, and the absence of any possible 'secondary effect' that these insecticides would have had on non-target pests, has placed the aim of pest control on secondary insects such as aphids, leafhoppers and stainers, while taking predatory numbers into account. Secondary pest numbers on Bt-cotton are presented, and the pegboard, or Cotton Calculus as a scouting tool is discussed, as part of an Integrated Pest Management approach on Bt-cotton.

Keywords:*Bollworms, secondary pests, scouting, Integrated Pest Management, Bt-cotton, South Africa*

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Reiterating the positive influence of surrounding vegetation in insect resistance management of Bt corn in a tropical country like the Philippines

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Ten years after its introduction in the Philippines, Bt corn planting had immensely increased around the Philippines. The new growth points in the last five years were in Central Luzon and in many parts of Mindanao (BPI, 2011). The hectarage and volume of production has significantly increased (BAS 2010, James 2010) coupled with the establishment of feed mills in those areas. Although climate change events are a threat, corn production is still constrained by pests and diseases. Bt corn has effectively regulated the most serious pest of corn, the Asian corn borer (ACB), Ostrinia furnacalis (Guenee). After a decade, there has been no report of development of ACB resistance in the original YieldGard hybrid that was introduced and in other Bt hybrids that followed. This paper reiterates the role of surrounding vegetation especially the alternate hosts (weeds and other economic crops) of ACB in insect resistance management of the original Bt hybrids and the second generation stacked Bt corn products. It discusses the role of other natural mortality factors that will create the variability in the ACB populations that will delay the development of resistance. This also takes into consideration the unique tropical setting of the country, the natural temporal refuge that can be harness if given due importance and the knowledge that most corn farmers only have at most a hectare to till..

Keywords:*Ostrinia furnacalis, Asian Corn Borer, Bt Corn, Alternate hosts, Insect resistance management*

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Terrestrial arthropod biodiversity in Bt corn fields: Assessing the impacts of Bt corn on non-target groups in the Philippines with special focus on uncommon or environmental indicator species

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The Philippines, being the first one to introduce Bt corn in Southeast Asia has been reaping the benefits of this technology. Hectarage and volume of corn production has increased along with farmers' income (BAS 2010, James 2010). Evidences from a series of fieldwork have shown that planting Bt corn has encouraged a wide range of arthropods in the corn field thus increasing diversity. This increase is probably a consequence of great reduction in pesticide use. Reduced pesticide use, or none at all, in turn, helps protect the environment and promotes better human health. This paper documents the arthropods associated with corn especially those ones not yet reported to occur in the Philippines. Previous studies documented mostly the pest species (e.g. Gabriel (1997). There was no record of other species that belong to other functional guilds or those species that serve as biological indicators or uncommon species. Relatively recent papers recorded parasitoids and predators associated with corn pests as part of the search for alternative control measures against major insect pests of corn in the past few decades. As farmers do not spray their corn fields anymore or if ever, very seldom, the resulting change in the environment, i.e. reduced toxic chemical concentrations, encourages diversity of arthropods in corn agroecosystems or communities.

Keywords:*Bt Corn, Pest management*

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O403

Effectiveness of transgenic bt cotton on the non-target sucking insect pest's community

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The transgenic Bt cotton can effectively control specific lepidopterous species but there is lack of resistance against non-target sucking insect pest's community. Among the non-target, sucking insect pests, cotton whitefly, jassid, thrips aphid and cotton mealy bug are the most important in Pakistan. These are very destructive pests during seedling and vegetative phase of cotton. A little attention is given on the non-target, sucking insect pest's community in Bt cotton as most of the studies focus on major target lepidopterous pests. So, the sucking pests have become a more significant part of pest complex in Bt cotton throughout the world. It is therefore, the study was planned to measure the effectiveness of transgenic Bt cotton against sucking insect pests community under field conditions. The transgenic Bt-cotton line, "IR-FH-901" was compared with its parent non-transgenic, conventional cotton cultivar, "FH-901" under sprayed and unsprayed conditions. The experimental fields were laid out in a Randomized Complete Block Design (RCBD) consisting of four treatments each with four replications. The results revealed no significant differences in population density of these non-target sucking pests between Bt and non-Bt cotton. Regarding sucking pests management no significant differences were found in insecticide applications between Bt and non-Bt cotton. So, for the long term implementation of Bt cotton as a component of IPM, it is important that such varieties should be transformed with Bacillus thuringiensis (Bt) toxin genes, which also have some resistance traits against these non-target sucking insect pests to reduce the number of insecticide applications.

Keywords:*Bt cotton, Population density, Sucking pests, Pakistan,*

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O403

Effectiveness of the Bph3 resistance gene against planthoppers in the Philippines

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Since 2002, frequent and severe outbreaks of the brown planthopper (Nilaparvata lugens)(BPH) have occurred on rice throughout Asia. One theory suggests that these new outbreaks have resulted from a general increase in susceptibility of rice varieties throughout the region. Evidence indicates that resistance in rice with the Bph3 gene has remained relatively stable. We examined rice varieties with Bph3 in the Philippines for their resistance against BPH. We also investigated factors that may contribute to a decline in resistance of these varieties during normal plant growth, and the effects of planthopper adaptation to resistant varieties. Rice varieties deemed to contain the Bph3 gene were variably resistant to BPH in the Philippines. Some varieties (i.e., IR60 and IR62) were highly resistant to the hoppers as determined from several bioassays. Resistance in IR62 was stable throughout plant growth increasing in effectiveness as plants aged. In one bioassay, resistance declined under high nitrogen levels, but these effects were inconsistent. Virulent BPH (that had adapted to IR62) were capable of feeding on all varieties with the Bph3 and bph4 genes. We are continuing to examine the effects of several classes of insecticide on resistance bestowed through Bph3. Evidence so far suggests that increased susceptibility in the field could result from both adaptation by hoppers to long-term deployed resistance genes and poor management of varieties with currently effective genes.

Keywords:*Brown planthopper, rice, resistance, adaptation*

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Evaluation of fumigant toxicity of essential oils from three medicinal plants against *Callosobruchus maculatus* adults (Coleoptera: Bruchidae)

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Fumigant toxicity of essential oils from Satureja hortensis (Lamiaceae), Teucrium polium (Lamiaceae) and Foeniculum vulgare (Apiaceae) were evaluated against adults of cowpea seed beetle, Callosobruchus maculatus exposed for 24h. Essential oils were obtained by hydro-distillation using a Clevenger-type apparatus. Experiments were conducted at 28±2°C and 60±5% R.H. in dark condition. Gas chromatography coupled with mass spectrometry was used to identify the main components of the essential oils. The principal constituents of F. vulgare oil were E-anethole (60.61%) and fenchone (12.14%) and those of S. hortensis oil were carvacrol methyl ether (50.13%) and thymol (26.77%). Teucrium polium oil mainly consisted of piperitenon oxide (21.72%), α-pinene (11.33%) and carvon (11.29%). All essential oils showed high fumigant activity against C. maculatus adults. The results indicated that mortality of 1-day-old adults increased with oil concentration. Males were more susceptible to oils than females. The essential oil of F. vulgare proved to be the most potent toxicant with the LC50 values of 21.43 and 29.6 µlL-1 air against males and females, respectively. The LC50 values of S. hortensis oil were 74.17 and 156.73 µlL-1 air and the counterpart values of T. polium oil were 60.1 and 80.93 µlL-1 air against males and females, respectively. The results demonstrated that the essential oils of these plants can be considered as the potential protectants against cowpea seed beetle.

Keywords:*Essential oils, Satureja hortensis, Teucrium polium, Foeniculum vulgare, fumigant toxicity*

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Monitoring of bifenazate and etoxazole resistance in two-spotted spider mite, *Tetranychus urticae*

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The two-spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae), is one of the most important pest species devastating many horticultural and ornamental crops and fruit trees. Difficulty in managing this mite is largely attributed to its ability to develop resistance to many important acaricides. Two strains were collected from the rose greenhouse in Chillgok and Buyeo in December 2000, respectively. These population has been selected for eleven years with bifenazate or etoxazole (over 500 times), and increased 856- and over 5,000,000-folds in resistance as compared with susceptible strain, respectively. Bifenazate- and etoxazole-resistant strains were both shown to be maternally inherited. By analyzing the mitochondrial cytochrome b (cytb) sequence, G126S point mutation was detected in bifenazate-resistant strain. Two molecular detection methods, quantitative sequencing (QS) and PCR amplification of specific alleles (PASA) were well detected specific G126S point mutation. However, point mutation of cytochrome c oxidase subunit I (COX1), COX2, COX3, cytb, NADH dehydrogenase subunit 1 (ND1), ND2, ND3, ND4, ND5, and ND6 gene was not detected in etoxazole-resistant strains. Thus, point mutation of etoxazole should be studied continually and G126S point mutation in the mitochondrial cytb is available molecular detection marker for selection of bifenazate resistance

Keywords:*Tetranychus urticae, bifenazate, etoxazole, point mutation*

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DuPont™ Cyazypyr™ insect control (DPX-HGW86, cyantraniliprole,): Product attributes that Influence effective pest control and crop improvement

Isaac Billy Annan

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DuPont™ Cyazypyr™ (cyantraniliprole, DPX-HGW86), is the second molecule of the anthranilic diamide chemistry (IRAC Group 28 insecticides) to be commercialized, but the first active ingredient that shows excellent efficacy on a cross-spectrum of important pests of agronomic crops. Pests that are well controlled include whiteflies, psyllids, aphids, planthoppers, leafhoppers, Lepidoptera, weevils, beetles, sawflies, fruit flies and leafminers. But the product exhibits all these unique features, while still conserving beneficial arthropods. Cyazypyr™ is effective on multiple life stages of pests, and thus ensures effective control of damaging pest populations. Cyazypyr™ is selectively active on the insect ryanodine receptor site, causing rapid impairment of muscle function which in turn results in rapid cessation of feeding, and impairment of locomotion, mating and other muscle-controlled insect activities. The rapid feeding cessation ensures that plants are protected from the direct feeding damage by pests, and also in reduced incidence of pest-vectored plant pathogens that would adversely impact growth and yield performance of crop plants. Key physico-chemical properties such as good solubility in water and ideal Log Kow confer excellent translaminar and xylem translocation properties on products powered by DuPont Cyazypyr™. Overall, the novel mode of action, unique cross-spectrum pest control feature, outstanding plant protection, preservation of beneficial arthropods, excellent translaminar and systemic movement, lack of cross resistance with other known existing products, among other product attributes, make Cyazypyr™ an ideal product to complement IPM and IRM programs in plant protection and crop production systems.

Keywords:*Cyazypyr™, HGW86, cyantraniliprole, ryanodine receptor, translaminar, xylem translocation*

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DuPont™ Benevia™ (DPX-HGW86, Cyazypyr™, cyantraniliprole): Control of *Idioscopus clypealis* on mango in the Philippines

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Benevia™ (DuPont™ Cyazypyr™ 100 OD) is the third diamide compound (IRAC Group 28 insecticides) to be commercialized. Benevia™ and other products powered by DuPont™ Cyazypyr™ show a cross spectrum activity on key sucking, chewing and rasping arthropod pests on a broad range of crop groups, including large fruits, small fruits and other tree crops, vegetables. Mango, Mangifera indica L., is a very important crop in the Philippines, and is considered to be the national fruit. The mango hopper, Idioscopus clypealis Lethierry (Hemiptera: Cicadellidae), is considered as the most serious pest of mango in the Philippines. It causes damage to developing panicles, abortion of flowers, dropping of fruits and ultimately severe reductions in mango yields. Other important pests include the oriental fruit fly. Benevia™ shows excellent controls of those two important pest groups, among others, and also provides outstanding plant protection. Benevia™ has no adverse effect on the predator and parasitoid natural enemies of pests, and the product also controls pest populations that are resistant to other insecticide products. Therefore, Benevia™ has an excellent fit in integrated pest management (IPM) and insecticide resistance management (IRM) programs. Data on the control of Idioscopus clypealis and of crop performance will be discussed.

Keywords:*DuPont™ Benevia™, Cyazypyr™, cyantraniliprole, anthranilic diamide,*

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Sulfoxaflor: A Novel insecticide for management of brown planthopper on rice

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Sulfoxaflor, discovered by and proprietary to Dow AgroSciences, is a member of a new chemical class of insecticides, the sulfoximines. Sulfoxaflor is being developed for use in all major crop groups globally, including cotton, soybeans, cereals, rice, citrus, leafy and fruiting vegetables, cole crops, grapes, apples, and many other crops. Sulfoxaflor controls economically important and difficult-to-control sap-feeding insect pests such as tarnished plant bugs, many species of aphids, jassids, whiteflies, planthoppers, and certain scales. A multi-year, multi-location field trial program was conducted in countries such as China, India, Japan, Thailand, and Vietnam, and results showed that sulfoxaflor had an excellent fit into the rice market for management of brown planthopper (Nilaparvata lugens), a key pest in all major rice-growing regions. Sulfoxaflor provided commercial levels of efficacy against this pest at low use rates (50-100 gai/ha). It was also fast acting (knockdown at 1-2 days after application), with extended residual control of up to 21 days after application. Due to its novel chemistry, distinct mode of action and lack of cross-resistance with a variety of other insecticides, sulfoxaflor will be a valuable rotational partner with other chemistries, play a key role in insect resistance management, and serve as an effective pest control tool for rice growers.

Keywords:*Sulfoxaflor, Insecticide, Brown Planthopper, Rice*

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Spinetoram, a new spinosyn insecticide for managing key insect pests of rice

Jim Xinpei Huang[1], Jim Dripps[2], Samanwong Somsak[3], Lakshmipathi Srigiriraju[4], Peng Wang[5], Jee Hwan Yi[6], Alisa Yu[7]
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Spinetoram is a new active ingredient in the spinosyn class of chemistry and is created via two chemical modifications to naturally-occurring spinosyns. These modifications increase insecticidal activity and photostability. Spinetoram was registered under the US EPA Reduced Risk Pesticide Initiative and received a US Presidential Green Chemistry Challenge Award in 2008. Spinetoram has been registered on rice in many countries for management of certain insect pests. The efficacy of spinetoram against leafroller (Cnaphalocrocis medinalis) and stem borers (Chilo suppressalis, Tryporyza incertulas) and other insect pests on rice has been demonstrated in field trials and under conditions of commercial use around the world. At 18-27 gai/ha spinetoram provided larval control and leaf protection, with a good knockdown at 1 day after application and residual activity of up to 14 days. In addition to high activity on young (1st-2nd instar) larvae of rice leafroller, spinetoram was also effective against large (3rd-5th instar) larvae of this pest. For rice stem borers, spinetoram at 30-36 gai/ha provided control in both early (dead heart) and late (white ear) stages of rice crop development. Spinetoram has the same unique mode of action as spinosad (IRAC Group 5), has low toxicity to mammals and other non-target organisms, and does not accumulate in the environment because it is rapidly degraded in soil and natural surface waters. It has minimal effects on most beneficial arthropods and has an excellent fit in rice IPM programs. Spinetoram is becoming a powerful new tool for integrated management of lepidopteran pests in rice.

Keywords:*Spinetoram, spinosyn insecticide, rice leafroller, stemborer*

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Response to Insecticides of spotted wing drosophila from British Columbia, Canada

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Spotted wing drosophila (SWD), Drosophila suzukii Matsumura, was first discovered in Canada in 2009 in the province of British Columbia, and has been identified as a pest with serious potential economic consequences. Studies were initiated to identify insecticides with acceptable efficacy and environmental profiles, and to generate data required to bring these materials to registration. Twelve materials representing diverse chemical classes and modes of action have currently been tested on male and female flies using a contact bioassay. 4-5 doses of insecticide were dissolved in acetone, introduced into glass vials, and the solvent evaporated while the vials were rotated to produce an even film on the glass surface. Mortality was assessed after 24 hours. Some materials had acceptable efficacy: malathion (organophosphate), Entrust (spinosad) and Delegate (spinetoram) (both spinosyn-based products), as well as the synthetic pyrethroids Ripcord (ctpermethrin) and Matador (lambda-cyhalothrin). Other materials had intermediate activity: Concept (imidacloprid + deltamethrin), tolfenpyrad, and Pyganic (pyrethrum). Additional materials were tested but did not show activity towards SWD. Significant differences in tolerance between male and female flies were observed for spinosad and spinetoram (spinosyn-based materials) and also for Pyganic, although wide confidence intervals in the Pyganic data make these results difficult to interpret. It was also observed that younger flies (24-48 hours as adults) were more tolerant to most materials than older flies 5 days of age or older. The requirement to use flies of consistent age as bioassay subjects is evident.

Keywords:*Drosophila suzukii, insecticide bioassays*

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Determination of acute toxicity (LD50s) of metaflumizone to *Helicoverpa armigera* ,Spodoptera exigua (Lepidoptera: Noctuidae) and *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) in lab bioassays

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The pests Helicoverpa armiqera in cotton, Spodoptera exiqua attacking many crops and Leptinotarsa decemlineata in potatoes have been developed resistance to different insecticides throughout the world and also in Greece, the determination of the LD50s for a new coming insecticide in the market is very important and necessary for establishment Base-line data for comparison of effectiveness among different compounds, comparison between different populations and early determination of possible beginning of resistance. The commercial formulation of the insecticide Alverde 24 sc (metaflumizone) was used in the Bioassays. Larvae L2 were exposed to different doses using the leaf-dip method. Cotton leaves for Helicoverpa armigera, sugar beet leaves for Spodoptera exigua and potato leaves for Leptinotarsa decemlineata (approximately the same size) were dipped into the test solution for 10seconds then allow to dry before put in to the dishes. Data were analyzed by probit analysis (finney). Assessment of mortality was estimated in 24h, 48h and 72h. LD50s for Helicoverpa armigera were correspondently 150, 94, 17.95 ppm of active ingredient, for Spodoptera exigua were 389,103, 23.24 and for Colorado potato beetle were 67.54, 14.73, 3.23. The maximum mortality was obtained in 72h which is the appropriate time for estimated the LD50s and for Colorado potato beetle for getting fast results the 48h is appropriate. The insects used in the bioassays were already resistant to some synthetic pyrethtoids. The toxicological profile, the low mammalian toxicity, the lack of cross resistance of metaflumizone to other insecticides making it ideal for use in integrated pest management systems.

Keywords:*Resistance, Metaflumizone, Spodoptera exigua, Helicoverpa armigera, Leptinotarsa decemlineata*

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Effect of DuPont Cyazypyr products on control of major pests of bulb vegetable crops in Asia

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DuPont™ Cyazypyr™ (cyantraniliprole, DPX-HGW86), is the second anthranilic diamide compound (IRAC Group 28 insecticides) to be commercialized. It has unique cross-spectrum efficacy on both sucking and chewing pests of rice and other agronomic crops throughout the world. Cyazypyr™ has excellent activity on a cross-spectrum of important crop pests, including key pests of bulb vegetable crops like onions, shallot and leek. The rapid cessation of pest feeding by Cyazypyr™ produces outstanding protection of onion from pest damage. Several field studies in the Asia-Pacific region indicate that among the key pests that are controlled by the products powered by DuPont™ Cyazypyr™ include:Delia antiqua(onion maggot), Thrips tabaci(onion thrips), Frankliniella occidentalis(western flower thrips), Liriomyza spp.(leafminers) and Laphygma exigua(beet armyworm). While Cyazypyr™ products provide excellent control of pests, they do not show any adverse impact on predators, parasitoides and beneficial arthropods in general. Also, Cyazypyr™ products are able to control pest populations that are resistant to other insecticides with different modes of action. Therefore, Cyazypyr™ products are very important in intergrated pest management (IPM) and insecticide resistance management (IRM) programs. Examples of field data will be presented.

Keywords:*DuPont, Cyazypyr, cyantraniliprole, HGW86, onion, shallot, leek, thrips, beet armyworm, Liriomyza sp*

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O404TU10

IPM

O404

Effect of the extracts of *Embelia ribes* Burm. f. (Myrsinaceae) leaf on a nontarget organism, Lynx spiders: *Oxyopes lineatipes* C.L. Koch (Araneae: Oxyopidae)

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Spider is one of common fauna in agroecosystem and plays an important role as the generalist pest control agent. In order to investigate the potential use of the new natural product in pest control, Oxyopes lineatipes C.L. Koch was used as nontarget organisms, since it was the dominant species found in physic nut plantation. The plant extracts of Embelia ribes Burm. f. (Myrsinaceae) leaf by fixed-bed contacting method with hexane was evaluated together with two commercial pesticides; amidine (a synthetic acaricide) and azadirachtin (another natural product). The extraction was dissolved into four concentration levels (0.616% w/v; 1.907% w/v; 2.748% w/v and 3.5 % w/v), with 10% ethanol. Commercial pesticides, amidine and azadirachtin, were dissolved in water into 0.4 % w/v and 0.0025 % w/v, respectively. Water and 10% ethanol were served as control solution. Within 2 hours in laboratory studies, 83% of O. lineatipes were poisoned by 50-$\mu\ell$ sprayed of Amidine, but neither by 0.616% E. ribes plant extracts, azadirachtin nor water. However, the higher concentrations of E. ribes plant extract resulted in higher mortality of O. lineatipes than 50% of total trials but within the longer period of time; 116 hours, 103 hours and 10 hours, respectively. All of O. lineatipes died after contacted with amidine for 21 hours, but some spiders still were alive in all other treatments at the end of an experiment (116 hours). The LC50 of this plant extracts were 1.38 and 1.27 % w/v at 24 and 84 hours, respectively.

Keywords:*Lynx spiders, plant extracts, Embelia ribes, Amidine, Azadirachtin, nontarget, LC50*

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O404

Lethal effects of four insecticides on different developmental stages of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) in laboratory conditions.

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The common green lacewing Chrysoperla carnea Stephens is a general biocontrol agent of several insect pests in greenhouses. The lethal effects of four compounds including imidacloprid, lufenuron, thiametoxam and thiodicarb on eggs, 1st instar larvae and adults of C. carnea were studied in laboratory conditions (25±2 ºC, 60±10% RH and 10-h photo phase). Dipping bioassay tests were used for eggs and residual contact method for larvae and adults. Positive relationships were detected between the concentrations of insecticides and mortality rates of various stages. However, there were considerable variations in toxicity of insecticides. Thiodicarb had no effect on eggs, whereas thiametoxam with the LC50 value of 1.90 µg ai L-1 showed the highest ovicidal activity. On larvae, thiametoxam was the most toxicant (LC50= 0.55 µg ai L-1) and lufenuron proved to be the least (LC50 = 44.02 µg ai L-1). The results of adult experiments indicated that males were more susceptible than females to all insecticides except Lufenuron to which no lethal effect was observed. Thiametoxam proved to be the most potent toxicant against males (LC50= 8.83 µg ai L-1) and females (LC50= 73.05 µg ai L-1). The use of thiametoxam should be carefully evaluated if employed in combination with C. carnea in IPM programs. Imidacloprid and thiodicarb are moderately harmless and lufenuron considered being safe for C. carnea.

Keywords:*Chrysoperla carnea, imidacloprid, lufenuron, thiametoxam, thiodicarb*

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O404

Investigations on the effects of two different plant extracts on the green peace aphide [(*Myzus persicae* Sulzer) (Hom.: Aphididae)]

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Green peach aphid [(Myzus persicae Sulzer) (Hom.: Aphididae)] is a very important pest worldwide, causing serious damage to vegetables, flowers and fruit crops. Green peach aphid is also an economic pest in Turkey. The use of plant extracts as an alternative pesticides for control of insects is becoming important. A great deal of research has been carried out in this area in recent years. The efficacy of pesticides extracted from two different plants such as Allium sativum L. (Amaryllidaceae) and Capsicum annum L. (Solanaceae) were tested as alternative insecticides. The effects of extracts with ethanol obtained from two different plants on Green peach aphid were investigated. Bioassays to were tested by two different methods determine the effects of varying concentrations. Experiments were performed using 3 cm diameter leaf disk from unsparayed the radish plants [Raphanus sativus L. (Brassicaceae)]. Experiments were carried out using (3cm diameter) leaf disks of R. sativus. The effects of four concentrations of extract %1, 3, 6, 12 were studied. All of experiments were repeated 10 times. As a result of the investigation, in leaf dipping method; the extract of C. annum %12 concentration showed the highest mortality in nymph and adult stages. There was a not significant difference on the mortality between leaf dipping and direct leaf spraying method when compared. The research undertaken in the laboratory at the Ankara Plant Protection Central Research Institute in 2010 tested the insecticidal effect of two plants extracts against Green peach aphid.

Keywords:*Green peach aphid, Allium sativum L. Capsicum annum L. extract, insecticidal effect*

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O404

Semi-field and field evaluations of spatial repellents for mosquitoes

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A spatial repellent is a chemical compound that in the vapor phase can produce repellency at a distance, significantly reducing the number of target species in a defined area over a designated period of time. Several compound classes have been reported to produce spatial repellency of Aedes aegypti, including essential oils, such as citronellal and geraniol, pyrethrums and synthetic pyrethroids. Several commercial products have been produced utilizing these compounds. Although many of these commercial products appear to show promise, they have not been adequately evaluated in peer-reviewed studies. The studies reported in this presentation were conducted in attempt to help fill that knowledge gap. Protocols were developed to evaluate these commercial products and other candidate compounds in laboratory olfactometer, semi-field and field studies against laboratory reared and/or natural populations of mosquitoes. It was determined that these compounds have both mortality and repellency properties. `Olfactometer studies emphasized screening candidate essential oil compounds; semi-field and field studies emphasized evaluations of an increasing array of commercial products, which contain active ingredients such as metofluthrin and allethrin.

Keywords:*spatial repellent*

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O404

Immunochemical techniques: Effective tool for pesticide residue detection

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Chlorpyrifos, being successfully detected by GC, LC and HPLC with great sensitivity and reliability. However, these are typically burdened with time-consuming sample preparation steps, skilled analysts, low detection level, high expenditure and false positives. Hence, immunochemical techniques supplement traditional analytical methods in an ideal way because they are extremely fast, sensitive, simple and inexpensive. The most important types presently used for environmental analysis are immunoassays, immunosensors, immunochromatography, and immunolabeling. The progress of development of new immunoassays and related immunotechnologies requires the availability of antibodies with the desired affinities. A critical step in the production of monoclonal antibodies is the development of efficient screening procedures to identify suitable hybridoma lines. The usefulness of immunomagnetic cell separation for the screening of hybridomas is based on the expression of surface receptors resembling the secreted antibodies on the membrane surface of hybridoma cells. Producers are tagged by the target molecule covalently linked to magnetic beads and subsequently removed by permanent magnet. Immunomagnetic beads offer an alternative to conventional screening with immunoassays as it can solve the problems like much time-consumption, escaping detection or loosing valuable cells. This technique is based on tagging, surface receptors of hybridoma cells with binding properties of the secreted antibodies by magnetic beads, which are coated with hapten conjugates. In spite of the considerable progress in hybridoma technology, antibody development has entered an entirely new domain because of the potential of recombinant antibodies. This technology will eventually allow faster production of antibodies with stepwise abandonment of animal experiments.

Keywords:*Chlorpyrifos, ELISA, Insecticide, Immunoassay, Immunomagnetic beads*

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Studies on the evaluation of new chemistry insecticide (Spinosad) and some plant extracts against *Rhyzopertha dominica* (Fab.) (Bostrichidae: Coleoptera)

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The efficacy of plant products onion and garlic, and new chemistry insecticude spinosad against Rhyzopertha dominica was studied under the laboratory condition of 32±2C and 70±5% R.H. following completely randomized design. Experiment was carried out on direct mortality. Expermental jars were washed cleaned and dried carefully to avoid any possibility of pathogenic infestation. Three concentrations of spinosad, onion and garlic such as 0.50, 0.75 and 1.00% were mixed in 100gm of fresh sterilized wheat grains for different exposure time (3, 6 and 9 days)and data collected was analyzed statically.The maximum mortality was found in case of spinosad. The remaining survival individuals were taken to assess population build-up. Population build-up data were recorded after 30 and 60 days of liberation of test insects. Observation on mortality basis and population build-up revealed that spinosad was the most effective at all concentrations. The minimum mortality and maximum population buils-up was in case of onion and garlic.

Keywords:*Plant extracts, spinosad, toxic, repellent, growth inhibitory, R. dominica*

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O404TU16

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Influence of DuPont™ Benevia™ (Cyazypyr™, cyantraniliprole) on insect pest management and plant protection in leguminous crops in Asia

Lu Ren
Dupont company, China

Benevia™ is a 100 g/L formulated OD product powered by DuPont™ Cyazypyr™ (DPX-HGW86, cyantraniliprole), which is the second anthranilic diamide compound (IRAC Group 28 insecticides) to be developed and commercialized by the DuPont Company. Benevia™ has shown excellent cross spectrum activity on key sucking and chewing insect pests of a broad range of agronomic crops worldwide. Benevia™ controls major pests of legumes crops in the Asia-Pacific region, including cowpeas, mung beans, snap beans, red gram and different types of peas. Among the important pest groups that Benevia™ controls include bean pod borer, vegetable leafminer, pea leafminer, leafhoppers, sweet potato whitefly, cluster caterpillar, cotton leafworm, beet armyworm, aphid and thrips. Benevia™ shows the excellent pest control while still having no adverse impact on key species of arthropod predators and parasitoids that contribute significantly to pest control. Therefore, the product will be a centerpiece of integrated pest management (IPM) programs. Other important attribute is the rapid cessation of pest feeding by Benevia™. This results in the protection of plants from direct damage from pest feeding, and also in reductions of vectoring of plant pathogen by pests. These include cowpea aphid-borne mosaic virus (CabMV), which is transmitted by the cowpea aphid. The benefits are achieved when the product is applied at the early stages of pest infestations before populations reach damaging levels. Benevia™ controls pests that are resistant to other insecticide products, therefore it will become a valuable rotational partner in insecticide resistance management (IRM) programs. Field data will be presented.

Keywords:*DuPont™ Benevia™, Cyazypyr™, cyantraniliprole, anthranilic diamide, cowpea aphid-borne mosaic virus,*

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Effect of *Ferula assafoetida* L. essential oil on some physiological aspects of the common pistaciae psylla, *Agonoscena pistaciae* under laboratory conditions

Mahshid Sarnevesht[1], Hamzeh Izadi[2], Mohamad Amin Jalali[3]

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The common pistachio psylla, Agonoscena pistaciae Burckhardt and Lauterer (Hem: Psyllidae) is known as a key pest of pistachio orchards in Iran. In this study, effect of Ferula assafoetida essential oil (a toxic extract from stinking assa plant) was investigated by measuring glycogen, glucose, lipid and protein contents of the 5th instar nymph of A. pistaciae. The 5th instar nymphs dipped in insecticide for 3s. Treated nymphs allowed to air dry and then located on the pistachio leaves in controlled conditions (27±2 oC, 60±5 RH and 16:8 h L:D). The effect was studied in 24 and 48 hr after treatment. Low molecular weight carbohydrate (glucose) as well as glycogen and lipid contents were measured by methods described by Warburg and Yuval (1996). Protein content of whole body was measured by Lowery method (Lowery et al., 1951). Glycogen content of treated nymphs 24 hr after treatment (19.29 mg/g fresh body weight) and 48 hr after treatment (7.7 mg/g fresh body weight) was significantly lower than control (9.15 and 17.12 mg/g fresh body weight).but no significant difference was observed between protein, lipid and glucose contents of treated nymphs and control (P>0.05). This study revealed that Ferula assafoetida is able to significantly decrease glycogen level of treated 5th instar nymphs in relation to control.

Keywords:*Physiology, Ferula assafoetida, Agonoscena pistaciae*

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O404TU18

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O404

Toxicity of acetamiprid and *Ferula assafoetida* L. essential oil against the common pistachio psylla, *Agonoscena pistaciae* under laboratory conditions

Mahshid Sarnevesht[1], Hamzeh Izadi[2], Mohamad Amin Jalali[3], Hadi Zohdi[4]

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The common pistachio psylla, Agonoscena pistaciae Burckhardt and Lauterer (Hem.: Psyllidae) is known as a key pest of pistachio orchards in Iran. Chemical control is the common method for the control of this pest, with growing concerns about unwanted impacts of conventional chemicals, investigations on compounds with few environmental side-effects such as plant extracts for pest control receives increasing attention. In this research, susceptibility of the 5th instar nymphs of A. pistaciae to F. assafoetida essential oil, a toxic extract from stinking assa plant, and acetamiprid, a neonicotinoid insecticide, was investigated. Probit analysis of concentration-mortality data conducted to estimate the LC50 values of the test chemicals. The 5th instar nymphs of A. pistaciae dipped in different concentrations of acetamiprid (0.1, 0.15. 0.2, 0.25 and 0.3 g/ml) and F. assafoetida essential oil (3, 4, 5, 6 and 7 ppm) for 2 seconds. Treated nymphs were then allowed to air dry and then located on the pistachio leaves in controlled condition (27± 2°C, 60±5 RH and 16:8 h L:D). The LC50 value was estimated 0.3 g/ml for acetamiprid and 6 ppm for F. assafoetida essential oil after 72 hr. Our results showed that F. assafoetida in comparison with acetamiprid is more effective and toxic against 5th instar nymphs of A. pistaciae.

Keywords:*Agonoscena pistaciae, Acetamiprid, Ferula assafoetida*

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O405TU01

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O405

Gonipterus platensis (Coleoptera: Curculionidae) - Present status and control strategies in colder regions of Portugal

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The eucalyptus snout weevil Gonipterus scutellatus Gyll. (Coleoptera: Curculionidae), as recently demonstrated, is a complex of cryptic species with different distributions. The eucalyptus snout weevil present in the Iberian Peninsula was further identified as G. platensis, originate from Tasmania. The egg parasitoid Anaphes nitens (Hymenoptera: Mymaridae), originate from South Wales, is the only biological control strategy extensively used worldwide to control this weevil complex. However, the parasitoid fails to effectively control G. platensis in the colder areas of the Iberian Peninsula such as in mountain regions, of the northern and centre of Portugal. Our results on egg parasitism rate, tree defoliation and wood loss following altitudinal gradients show a consistent decreasing of parasitism rates and increase defoliation damage with elevation. Consequently, timber production losses increase steeply with elevation, reaching 90% of wood loss above 700 m. A linear decrease of the parasitism rate with the decrease of the maximum temperature of the winter months is further observed. Therefore, a climatic mismatch between the parasitoid and the weevil is hypothesized. As a result, alternative control strategies are being investigated. We present results on the weevil preference and performance on Eucalyptus species alternative to E. globulus. Further, we discuss the possibility of using natural enemies of G. platensis from Tasmania in a classical biological control program.

Keywords:*Eucalyptus, Defoliation, egg parasitoid, Anaphes nitens, Biologicol control, Wood loss*

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Humidity-controlled cold storage of the predatory mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae), an important natural enemy of spider mites

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The major obstacles to the successful implementation of biological control are the high cost and poor availability of natural enemies. However, by developing an effective storage method, reduction in the costs and increase in the availability can be achieved. This study aimed at developing such a storage method by controlling humidity and selecting a specific life stage of the predatory mite Neoseiulus californicus, an important natural enemy of spider mites. Larvae, protonymphs, deutonymphs, unmated adult females, and mated adult females of N. californicus were stored at 5°C and relative humidity (RH) of 100% or 80%. The survival period was significantly longer at 100% RH than at 80% RH for all the life stages. Mated adult females exhibited the longest survival. Post-storage reproduction of the stored mated adult females was not affected by storage for up to 30 days. These results showed that mated adult N. californicus females can be stored at 5°C and 100% RH for 30 days without any degradation in post-storage quality.

Keywords:*Biological control, Cold storage, Life stages, RH*

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O405TU03

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O405

Dust in apple orchards causes mite outbreaks: Fact or fiction?

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Five apple orchards exposed to varying amounts of dust were used. In each orchard 25 evenly spaced trees were identified as sample trees. One leaf was taken from the periphery and one from the inside of each tree. Adult phytophagous and predatory mites were counted on these leaves, which were placed in a paper bag and transported to the laboratory where all stages were counted microscopically. One leaf was taken from each tree for determining the amount of dust. They were placed individually in paper bags. In the laboratory the dust was washed off and filtered through pre-weighed filter paper, which was dried and re-weighed. The difference in weight before and after filtering represented the weight of dust on the leaves. The area of each leaf was determined, enabling estimation of the amount of dust in terms of mg cm-2. The season with the most dust had the least number of mites and visa versa. Therefore, seasons during which there is a lot of dust do not necessarily result in mite outbreaks. There was no relationship between the amount of dust on leaves from different orchards and mite numbers, suggesting that dusty orchards do not harbour high mite population levels. Of the 15 correlations between the amount of dust on individual trees and the number of mites on these trees, one was highly significant and one was marginally significant. Therefore, trees with a lot of dust do not always harbour more mites than trees with less dust.

Keywords:*Mites, dust, apples*

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O405

Are neonicotinioid insectides which partially replaced organophosphate and carbamate insecticides in U.S. tree fruit safer to native pollinators and biological control?

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The U.S. Food Quality Protection Act (FQPA) of 1996 has or is in the process of eliminating many of the organophosphate (OP) and carbamate broadspectrum insecticides that eliminated most biological control and many of the native pollinators in the tree fruit orchards of the eastern U.S. Some of the U.S. Environmental Protection Agency classified reduced-risk and OP replacement insecticides that have replaced them are target specfic and are promoting conservation of biological control and possibly native pollinators. Is the widespread use of neonicotinoids in orchards a step forward towards ecologically based IPM? Biodiversity and abundance data from various arthropod taxa, including bees, collected in replicated trials in commercial orchards over 3 years, from sentinel egg masses placed in orchards, bio-assays and field residue analysis from orchard applications will be presented.

Keywords:*Pollinator, neonicotinioid, organophosphate, Biodiversity, IPM, Reduced-risk pesticides*

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O405

Phanerotoma philippinensis, a potential parasitoid for managing legume pod borer, Maruca vitrata

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[1]AVRDC - The World Vegetable Center East and Southeast Asia, Thailand, [2]AVRDC - The World Vegetable Center, Taiwan

Legume pod borer, Maruca vitrata, is a serious production constraint on yard-long bean, which is grown on approximately 7% of the total vegetable production area in Southeast Asia. M. vitrata can cause up to 80% yield loss on various host plants. Pod borers are difficult to control with chemical pesticides because they burrow into the pods, where they are protected from the pesticide spray. Parasitoids could play a significant role in suppressing borer insects including M. vitrata even when the pests are concealed under or inside plant parts. A systematic effort to identify the most effective parasitoids of M. vitrata in Thailand identified Phanerotoma philippinensis (Braconidae: Hymenoptera) as a major parasitoid. In a monitoring survey conducted during 2011 in Nakhon Pathom, field parasitism up to 21% was recorded. P. philippinensis is an egg-larval parasitoid; the females attack the egg stage, and the adult parasitoid emerges out from the second instar larvae of M. vitrata. A female parasitoid can parasitize about 10-50 eggs in three hours. However, successful emergence of parasitoid adults from the parasitized eggs had an inverse relationship with the host egg density. P. philippinensis females preferred one-day-old eggs of M. vitrata. This parasitoid is amenable to mass-culturing under insectary conditions using M. vitrata eggs as the host. The potential for using P. philippinensis together with different chemical and biopesticides in an integrated pest management strategy will be discussed.

Keywords:*Phanerotoma philippinensis, Maruca vitrata, egg-larval parasitoid*

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O405TU06

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O405

Toxicity of insecticides to *Aphelinus mali*, the parasitoid of woolly apple aphid

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The parasitoid Aphelinus mali is vital for the successful biological control of woolly apple aphid (WAA; Eriosoma lanigerum) in New Zealand apple orchards. WAA is normally well controlled by A. mali, but there were serious outbreaks in the 2007-08 season, which coincided with the introduction of a new insecticide for codling moth control to satisfy residue restrictions. Bioassays incorporating field application of four insecticides and exposure of A. mali to 0 to 15-day-weathered residues on leaves were used to determine toxicity. Fresh residues of spinetoram, spinosad, thiacloprid and spirotetramat had high, moderate, low and no toxicity respectively as measured by adult mortality. The neonicotinoid thiacloprid also caused paralysis and twitching of the treated insects, resulting in high toxicity as measured by the inability of A. mali to walk. Spinetoram was more persistent than spinosad, resulting in moderate toxicity 7 days after application, whereas spinosad was benign to A. mali at this time. Applications of one eighth field rate of spinetoram had similar residual toxicity to the full rate of spinosad. We postulate that repeated applications of spinetoram were the primary cause of the observed WAA outbreaks, in conjunction with other disruptive insecticides. Spinetoram does not control WAA but is toxic to A. mali leading to widespread severe outbreaks of WAA. Strong dependence on a single biocontrol agent made this IPM system vulnerable to disruption by toxic pesticides. Minimising the use of pesticides toxic to A. mali should benefit the sustainable management of WAA in New Zealand apple orchards.

Keywords:*woolly apple aphid, Aphelinus mali, off target toxicity, Eriosoma lanigerum, insecticides*

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O405

Biological characteristic and selection of low toxic of pesticides to native predator, *Chrysoperla nipponensis* (Okamoto) (Neuroptera:Chrysopidae) for the control of mealy bugs at pear Orchard.

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The mealy bug is known as a pests on bark, stem and leave of woody and herbaceous plants, including pear. Mealy bugs are soft-bodied creatures, but they secrete a powdery wax layer over themselves. It makes so difficult to chemical control. The development of new effective native predator against the mealy bugs is of great interest. We found that the lacewing had a potent control efficacy against mealy bugs. The identified DNA sequences indicate that this lacewing species is Chrysoperla nipponensis (Okamoto). C. nipponensis has been commonly used with biological control agents for control of several kinds of pests(aphids, whitefly, thrips, mites and mealy bugs) in agroecosystems, because of its abundance and broad habitat range. The toxicities of 12 commercial pesticides(8 insecticides and 4 fungicides) which have been used in the domestic market were evaluated to C. nipponensis at the recommended concentration. Among 8 insecticides tested, Imidacloprid 10%, Thiamethoxam 10%, Acetamiprid 8%, Methidathion 40%, Acequinocyl 15%, Clothianidin 8% and Deltamethrin 1% showed low toxicity to C. nipponensis. All fungicides tested were very low toxicity. It may be suggested from these results that 7 insecticides and 4 fungicides could be incorporated into the integrated pseudococcidae management system with C. nipponensis on pear orchard.

Keywords:*Lacewings, Chrysoperla nipponensis, Mealy bugs, Pear, Pesticides*

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Insects can enhance the biocontrol potential of pathogens against weeds: A case study on aquatic weed, waterhyacinth in South Africa

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While six arthropod agents have been released for biological control of the invasive aquatic macrophyte, waterhyacinth, Eichhornia crassipes Mart. Solms-Laubach (Pontederiaceae) in South Africa, not much research has been done on the plant pathogens of the weed. An approach to enhancing the weed killing effects of pathogens is to facilitate their entry by biologically, physically, or chemically wounding the weeds. Indeed, even ordinarily incompatible pathogens can become infective on otherwise resistant weeds when those plants are injured by various methods, including by insect feeding. Plant pathogens of waterhyacinth have been largely ignored in the country due to their erratic effect. During our recent studies we isolated some promising agents of waterhyacinth, including Alternaria eichhorniae, Acremonium zonatum, Bipolaris hawaiiensis and Fusarium spp. Although most of these isolates appeared severely damaging under controlled conditions their inability to serve as stand-alone replacements for chemical herbicides, has probably deterred their earlier commercialization efforts. Our studies showed potential increase in their pathogenicity in presence of waterhyacinth weevils, Neochetina spp and leaf-sucking bug, Eccritotarsus catarinensis, insect biocontrol agents of waterhyacinth. Thus the presence of six insect agents can be an advantage in enhancing the use of the native pathogens of waterhyacinth as effective biological control agents with their combined applications.

Keywords:*insect biocontrol agent, phytopathogen, weed, water hyacinth*

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Temperature effects on development of overwintering *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) from Korean apple orchards

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The oriental fruit moth (OFM), Grapholita molesta (Busck) (Lepidoptera: Tortricidae) is an important economic pest of stone fruits. The objective of the study was to determine the effects of temperature on the development of overwintering G. molesta larvae to adult in the laboratory. Five constant temperatures (15, 20, 25, 30, and 35ºC), with R.H. 75% and a photoperiod of 16L:8Dh were treated The developmental period significantly decreased with increasing temperatures; 53.8, 45.5, 28.5, and 20.7 days at temperatures of 15, 20, 25, and 30°C, respectively. The pupa did not survive at 35°C.The male developed faster than the female especially in larval stage. Linear and nonlinear regression models quantitatively described temperature-dependent development of Grapholita molesta. The developmental threshold temperature for cocooned larvae to adult was 7.3°C with the thermal constant of 490.19 degree days. The female ratio was not different to 0.5. . Morality was lower in 20-25°C treatments but higher in higher or lower temperature. At 35°C, mortality was almost 100%. During this study, we found that substantial proportion of collected overwintering cocoon (25±3.5%) was parasitized. Parameters from this study can be utilized for prediction of spring phenology of G. molesta in apple orchards.

Keywords:*Developmental period, lower developmental threshold, thermal constant, mortality, parasitized. sprin*

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Population management of invasive mealbug, *Phenacoccus solenopsis*(Tinsley) in cotton crop through different modules in Pakistan

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Phenacoccus solenopsis Tinsley is polyphagous invasive species which has devastated cotton crop in Pakistan in recent years. Studies were carried out on efficiency of parasitoid, Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae) against mealybug, P. solenopsis under field conditions at Center of Agriculture and Bio-sciences, Southeast and West Asia (CABI), Model Farm at Mirpur Khas, Pakistan. Two pest management modules, one practiced by farmer applying insecticides and another biological control module, using natural enemies' field reservoir (NEFR) were tested on cotton crop, 2010. Significantly high pest, parasitoid and predators population development were recorded in NEFR treatment compared with farmer's cotton. The seasonal mean population of P. solenopsis, parasitized pest mummies and predators were 27.40±0.6, and 19.10±0.20 per 10cm twig per plant and 5.46±0.30 per plant, respectively in NEFR cotton. At farmer's field significantly less parasitization of P.solenopsis and less incidences of hyperparasitism by hyperparasitoid, Promuscidea unfasciativentris on A. bambawalei were recorded compared with NEFR. The mean population of A. bambawalei recorded on farmer's cotton was 8.35±1.62, and in NEFR 15.75±1.54 parasitized mealybug mummies per 10cm twig per plant. The population of hyerparasitoid, P. unfasciativentris was 1.59 per twig per plant with 12.38% parasitization of A. bambawalei on farmer's field compared with 3.39 and 17.17 % on NEFR cotton crop. Economic analysis of different pest management strategies showed that the chemical control strategy of farmer gave more than double income compared to NEFR.

Keywords:*Phenacoccus solenopsis, Aenasius bambawalei,parasitoid*

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Diversity and distribution of natural enemies (predators and parasitoid) of bagworms (Lepidoptera: Psychidae) on various host plants in oil palm plantation

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Several species of predators and parasitoids were known to regulate the populations of oil palm (Elaies guineensis: Palmae) bagworms (Psychidae) in FELDA oil palm plantation in Perak, a northern state in Peninsular Malaysia. The abundance and diversity of these natural enemies on three species of host plants; Turnera sp. Cassia cobanensis and Antigonon leptopus was monitored at monthly intervals for 14 months in 2005 ? 2006. Seven hymenopteran and one hemipteran families were identified from 816 individuals collected. Chalcididae was the most dominant family (69.2%) and represented by three species of Brachymeria, B. lasus, B. lugubris and B. carinata. The abundance of natural enemies were significantly different among sampling periods (Kruskal Wallis X2=38.791, $p<0.05$) and host plants (Kruskal Wallis X2= 86.868, $p<0.05$). Most parasitoids including B. carinata and Dolichogenidae metasea preferred C. cobanensis and Turnera sp. over Antigonon leptopus ($z= 2207.5$, $p< 0.05$ and $z=4312$, $p=0.00$ respectively). No significant preference of the parasitoids on C. cobanensis and Turnera sp. was detected ($z= 10571$, $p>0.05$). High abundances of natural enemies were observed on C. cobanensis and Turnera sp due to the presence of stipules on their stems and flowers respectively that produce nectar as a food source for the insects. The plants also provided good shelters for the natural enemies. This study has shown the importance of host plants in oil palm plantation to support high abundance and diversity of natural enemies that would contribute to sustainable control of oil palm insect pests.

Keywords:*Bagworm, Parasitoid, Predator, Beneficial Plant*

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Response of cotton mealybug to biological, biochemical and chemical control tactics under semi-field and field conditions

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Two trials were carried out under semi-field and field conditions to evaluate the compatibility of native predators, Chrysoperla carnea larvae and adults of Brumus suturalis and the exotic predator Cryptolaemus montrouzieri in combination with neem oil for the control of cotton mealybug, Phenacoccus solenopsis. The assessments were based on mealybug control (scale 0-9) and percent recovery of predators at the end of the experiment showing conservation and colonization capability of the predator to local conditions. Both native predators Chrysoperla carnea and Brumus suturalis showed better control of the mealybug under semi-field and field conditions over the control and were also recovered at the end of the trials. In contrast to these, exotic predator, Cryptolaemus montrouzieri though proved to be the most efficient predator of cotton mealybug, Phenacoccus solenopsis under semi-field conditions but it failed to establish under field conditions and reducing mealybug populations and no recoveries were made of the predator in either of the treatments under field conditions. Maximum reduction in the population of mealybug was noticed in the insecticide treated plants. Our results show that application of neem oil followed by release of C. carnea larvae and B. suturalis adults can be used in swapping to synthetic insecticides for the safer management of mealybug on cotton.

Keywords:*Phenacoccus solenopsis, Chrysoperla carnea, Brumus suturalis, Cryptolaemus montrouzieri, neem oil*

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Orientation and feeding responses of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) adults to honey and sucrose

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Orientation responses of naive, unmated and unfed adults of Cotesia plutellae to honey and sucrose were tested in a T-shaped tunnel. Female wasps showed attraction, antennation and mouth parts extension to honey at a distance in response to its odor. Attraction of females to honey was innate because the wasps derived from sucrose-fed parents showed same level of attraction to honey as those derived from honey-fed parents. Hexane fraction of hexane-extracted honey attracted females, but did not elicit antennation and mouthparts extension. Aqueous fraction of hexane-extracted honey elicited latter two responses but no attraction. Males showed antennation and mouthparts extension responses but no attraction to honey odor. On allowing access to honey, sucrose or sucrose in the midst of honey vapor in feeding chamber, females reached honey directly without preceding random movements. But they took longer to reach sucrose after random movements. Males took longer than females to reach honey and much longer to reach sucrose after prolonged random movements. Sucrose in the midst of honey vapor was found and fed upon in almost same time as honey alone. Although a high percentage of wasps fed on honey, sucrose and sucrose in honey vapor, total duration of feeding of females on honey was longer than that on sucrose but it further extended when sugar was provided in the midst of honey vapor. Males fed on these foods for shorter durations than females. The study of using honey or its fractions in food supplements can be implemented for biological control program.

Keywords:*Cotesia, attraction, feeding response, honey odor*

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Biology of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and parasitism by *Psyllaephagus stenopsyllae* (Tachikawa) (Hymenoptera: Encyrtidae) in Baluchestan, Iran

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The Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Psyllidae), is a major pest of citrus causing damage and vector of citrus greening in south of Iran. In this study, the biological characteristics of each life stage of Diaphorina citri Kuwayama in laboratory conditions (25±1°C, 70±5% R.H. and a photoperiod of 14:10(L: D) hours and parasitism by Psyllaephagus stenopsyllae (Tachikawa) were investigated. The pre-oviposition period of D.citri was 3.9±0.47 days and the incubation period was 3.9±0.47 days. The average number of eggs laid per female was 230.25± 50.7 eggs. The mean of total nymphal period (1st instar to 5th instar) was 14.83±18.7 days. The mean longevity of male and female adults was 21.6±0.5 and 26.6±2.1 days, respectively. Mean parasitism of D.citri by P. stenopsyllae was 7± 6% in 2008.

Keywords:*Diaphorina citri, citrus, parasitism*

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Persist action of some toxic pesticides on *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae)

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Agricultural Research Corporation, Sudan

Parasitoids of the genus Trichogramma are distributed world-wide and play an important role as natural enemies. To enhance the role of these beneficial insects, the side effects of various pesticides should be tested. In this study the duration of harmful activity (persistence) of 5 harmful pesticides in laboratory tests was carried out. Potted plants were sprayed to runoff using field recommended dose of tested pesticides. Leaves from tested plants were then removed at different periods and Trichogramma adults were exposed to these leaves in standard exposure cages. The percentage reduction in parasitism relative to the control was then measured. Confidor and Dicrazol remained harmful {International Organisation for Biological Control (IOBC) - Class 4} even 24 days after treatments. Chess was moderately toxic. Aztec and Masi were slightly toxic 24 days after treatment. The relationship between parasitism rate and time for various treatments were measured during the study. It was observed that correlation coefficient (R2) showed a clear trend. For the control it was less than 0.01, and for harmful pesticides it was less than 0.9, whereas for the others it was more than 0.9.

Keywords:*Parasitoids, Trichogramma, Pesticides, Side effects*

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Effect of artificial nectars on survival of *Microctonus aethiopoides* (Hym.:Braconidae) parasitoid of *Hypera postica*(Col.:Curculionidae)

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Many parasitic wasps utilize floral resources, such as nectar to satisfy energy requirements for maintenance and reproduction. Floral nectar consists largely of carbohydrates (chiefly sucrose, glucose and fructose) and amino acids especially proline. The success of biological control is partly mediated by the longevity of beneficial insects and aavailability of nectar can improve the nutrition of parasitic insects, and thereby increase their longevity. Newly emerged wasps (<12h longevity) were feed singly whit 14 different solution presented on approximately 5 ×5 (mm) section of filter paper: the tap water (control), 2M glucose, 2M sucrose, 2M glucose+ fructose, 2M fructose, 2M clover honey, 2M sterile (heated) clover honey, water extracts of white clover leaf and flower and five combination of glucose (15%) and proline (0.05, 0.1, 0.25, 0.5, 5 %) to select the best of them. The experiment conducted in laboratory condition (20 ±1ºC, 14:10 L: D and 60±10% R.H.). Survival analysis was used to examine the effect of different diets on the longevity of M. aethiopoides. The product limit (Kaplan?Meier) method was used to estimate the survival distribution function in the analyses. The results indicated that in the male and female, between of carbohydrates, glucose have the most effect on longevity and also between of combinations of glucose and proline, the lowest concentrations (0.05 %) have a positive significant effect on longevity. While the highest concentration (5%) have a negative significant effect.

Keywords:*artificial nectars, Microctonus aethiopoides, Hypera postica*

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Effect of mass rearing of green lacewing, *Chrysoperla carnea* Stephens (Neu.: Chrysopidae) on growth rate index during multiple generations in

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Green lacewing, Chrysoperlacarnea Stephens is a polyphagous predator which used in inundation releases against a wide range of field and greenhouse insect pests, after mass rearing in insectarium. The main challenge in this method of biological control is the adverse effects of multiple reared generations of natural enemy under controlled conditions on its biological characters which may finally decrease pest control efficiency after field releasing. This experiment was designed and conducted in order to determine these adverse effects on a mass culture colony of green lacewing with collecting 570 adults from different regions of Golestan province. The stock was reared for 10 generations in insectarium of Agricultural and Natural Resources Research Center of Golestan. A mixture of yeast+sucrose (1:1) and eggs of the Angoumois Grain Moth (Sitotrogacerealella) were used as food for adults and larvae, respectively. Biological parameters including fecundity, pupalweight , percent of abnormal eggs,index growth ,length of egg,larva,pupa, preoviposition,adult and mortality of eggs,larva and pupal stage were measured for ten generations. Rates of adverse changes in these parameters were not equal. Deterioration start points with sharp decline for fecundity, pupal weight and percent of abnormal eggs and other changes were observed in 7th, 5th and 8th generations, respectively. Finally, in order to preserve predator vigor, it is recommended to limit insectarium cultures to 6th generation in similar conditions.

Keywords:*Chrysoperlacarnea, green lacewing, biological control, mass rearing*

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Sustainable management of *Spodoptera litura* by using *Bracon hebetor* and selective insecticides

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Armyworm, Spodoptera litura, is one of the most notorious polyphagous pests, attacking the cotton crop and hence depriving the farmers from getting good yields. The project under study, was, therefore, planned to develop some control strategies which are not only effective in their implementation, but also safer to the non-target organisms. For this purpose, a bio-control agent, Bracon hebetor (Say.) was used to control this notorious pest, along with the integration of some new chemistry insecticides throughout the cotton growing season. The insecticides under all the three conditions (Laboratory, semi-field and field) proved to be toxic for S. litura, but chlorpyrifos, profenofos, emamectin benzoate, spinosad, indoxacarb, methoxyfenozide and lufenuron proved to be highly toxic for the pest as the exposure time was extended. No insecticide in these experiments proved to be completely safe against the B. hebetor when the exposure time was extended but the above mentioned insecticides were comparatively safe than the remaining insecticides except chlorpyrifos and profenofos. The novel insecticides; emamectin benzoate, spinosad, indoxacarb, leufenuron and abamectin were safe for the parasitoid after short exposure time periods. These novel insecticides are therefore recommended for integration with the bio-control agent (Bracon hebetor). However, it is strongly recommended that the parasitoid should be released at least 24 hours after the application of these insecticides.

Keywords:*Sustainable Management, Spodoptera litura, Bracon hebetor, Selective Insecticides*

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O406

Azadirachtin based biopesticides influences insect head proteins in gram pod borer, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

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The demand of plant biopesticides have increased tremendously for integrated pest management and organic method of cultivation in arable crops because of harmful effects of synthetic pesticides. The azadirachtin, a tetranortriterpenoid obtained from Indian neem [Azadirachta indica A. Juss (Meliaceae)] is well known as potent phytogenic insect growth inhibitor to several insect orders namely Lepidoptera, diptera, orthoptera, hemiptera, coleopteran and hymenoptera etc. and commercialized extensively which have been found to influence the level of protein significantly in herbivores. The experimental result indicated that on average a 0.1 µg/ larvae dose of azadirachtin was found to reduce 30% in protein level up to 72 h in 7-day old larvae. However, the magnitude of reduction (23%) in 20 day-old larvae was found only 24 h after treatment in Helicoverpa armigera Hub. The results showed that azadirachtin decreased the total head protein of H. armigera in a dose-dependent manner. Besides, insect diet mixed with different azadirachtin-based formulations fed to fourth instars larvae of H. armigera also significantly ($P<0.05$) reduced the protein level. SDS-PAGE analysis of protein showed that azadirachtin suppressed 22, 26, 40 and 56 kDa head polypeptides in various treatments. Alternation in SDS polypeptide pattern was also observed with different modes of application in a dose dependent fashion. This information may be a novel basis of developing potential plant biopesticides based on screening protein estimation in relation to their bioactivities in future.

Keywords:*Azadirachtin, biopesticide, Helicoverpa armigera, polypeptide, head protein.*

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O406W02

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Natural plant compounds as insecticides for management of agricultural and veterinary pests

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Due to increased global awareness and concerns regarding the use of synthetic pesticides in general, as well as the direct and latent effect on the environment and severe insect population resistance problems, focus has shifted to alternative means of pest management. Among these strategies the use of natural plant derivatives as natural or botanical insecticides has emerged at the forefront of research. Several very successful botanical pesticides have been marketed thus far. At the moment, the worldwide demand for natural insecticides is increasing. Considering the ecological role of secondary plant products in plant-insect interactions, the strategy of isolating, identifying, and bioassaying these compounds seem to be an effective method of pesticide prospecting. The eventual aim of this study is to obtain viable moleculles for use as natural pesticides on several insect species of agricultural or veterinary importance. The active compounds of promising extracts will also be isolated and purified for accurate mapping. Initial bio-assays were conducted on stable flies, Stomoxys calcitrans (Diptera: Muscidae). These insects are fairly robust dipterans and important veterinary pests in their own right. The extracts of three promising plants, i.e. Zanthoxylum capense (Rutaceae), Agave attenuata (Agavaceae) and Dalbergia hupeana (Fabaceae), were examined for pesticidal efficacy. Efficacy trials were conducted on-site at the University of the Free State, with both Z. capense and A. attenuata emerging as very promising candidates for further testing. Currently, live colonies of various agricultural pests are being established, and further efficacy trials will be conducted as soon as colonies have become established.

Keywords:*Natural pesticides, Plant extracts, Pest Management*

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O406

Usage of Insecticide spraying equipment and protective measures by cocoa farmers in Nigeria

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The inappropriate use of cocoa pesticides and application equipment results in health and environmental hazard and development of insect biotypes. This study was carried out in three states of Nigeria using randomly administered questionnaires to thirty cocoa farmers in each state. The manual knapsack sprayer was found to be the widely used spraying equipment by the farmers. Despite their popular usage, only very few farmers (8% to 16.7%) used their graduated lid in the measurement of the insecticides. They rather preferred to use empty tin of evaporated milk (33.3% to 68%) or top cover of insecticide's bottle (24% to 50%) in the measurement of the insecticides into the knapsack. The farmers in all the selected states had different parts of the cocoa farms and/or plants that they preferred to spray and these ranged from the whole cocoa farm (29.2% - 40%) to part of the farm (16.7% -48%) to only affected trees (12% - 50%). Majority of the farmers (44% - 58.3%) did not comply with the use of necessary personal protective equipment (PPE) during spraying operations on their farms. A major challenge associated with cocoa farm spraying equipment as indicated by 70.8% to 80% of the farmers was high costs followed by lack of technical know-how to use the equipment as shown by 16.7% to 25% of the farmers. There is therefore an urgent need for massive training of the farmers on the practical use and maintenance of appropriate spraying equipment that are time saving and cost effective.

Keywords:*Cocoa farmers, insecticide application, personal protective equipment, knapsack sprayer, questionnai*

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O406W04

IPM

O406

Evaluation of novel acaricide, cyflumetofen for management of tea red spider mite, *Oligonychus coffeae* Nietner

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Tea is grown in 15 states of India among these Assam is top with 50.7% of total production followed by West Bengal with 22.1%, Tamil Nadu 15.9% and Kerala 8.3%. However, it is estimated that 15-20% of the total crop is lost annual from the ravage of pests. More than 300 insects and mites are associated with tea plants among them, red spider mite, Oligonychus coffeae (Nietner) is on the verge of the assuming the status of key pest on tea and along can damage more than 5-15% of the total crop in several specific geographical locations of West Bengal. To protect the crop, pesticides are in use since long back but pest species are now showing less susceptibility to some age old pesticides and also develop resistance. Hence, it has become important to search out new acaricide that will be selective in action against the mite, less toxic to beneficial arthropods, free for health hazards and also safe to environment. Studies were made to evaluate bio-efficacy and phytotoxicity of cyflumetofen a novel highly selective acaricide and causes paralysis and death, along with some new acaricides against tea red spider mite under field conditions. The experiment was carried out at terai region of West Bengal, India. Cyflumetofen 20%SC having good cidal effects on different stages of life cycle of tea red spider mite without showing any phytotoxicity symptoms on tea plants and safe for environment. So, it may be incorporated in IPM programme.

Keywords:*tea mite, cyflumetofen, management and Oligonychus coffeae*

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Insecticidal efficacy of *Beauveria bassiana* (Balsamo) Vuillemin mixed with diatomaceous earth against *Rhyzopertha dominica* F.

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The effects of mixing Beauveria bassiana (Balsamo) Vuillemin (1.7 x 104, 1.7 x 106 and 1.7 x 108 conidia kg-1 of grains) with diatomaceous earth (DE) formulation SilicoSec (100 ppm) against two populations of Rhyzopertha dominica F. (Coleoptera: Bostrychidae) were assessed. The trials were conducted on wheat grains at 30oC and 65% rh and the mortality of R. dominica adult was calculated 5, 10 and 15 d after exposure. The progeny production was observed from the treated units held for 60 d. Reseults revealed significant differences in adult mortality among populations and combined use of B. bassiana and DE increased adult mortality which was directly related with dose rate and exposure interval. The combined treatment was least effective against Bahawalpur population and most effective against Toba Tek Singh population of R. dominica. The reduced emergence of progeny was observed where B. bassiana and DE was combined at their higher dose rates. The data about the mycosis and sporulation revealed the maximum rate of mycosis and sporulation in cadavers of R. dominica where the low dose rates of B. bassiana were applied. The results of the present work suggest that the effectiveness of B. bassiana could be enhanced by the addition of DE, but the efficacy varies among populations.

Keywords:*B. bassiana, SilicoSec, R. dominica, progeny, mycosis, sporulation*

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Efficacy of safer insecticides against aphid on celery

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Celery plant (Apium graveolens) is cultivated for its fleshy leafstalks and seeds which yield essential oil. Aphids (Myzus persicae) suck sap from plant and reduce yield. Also they transmit viral diseases and contaminate celery produce with honeydew. Some safer insecticides namely Flonicamid 50 WG @ 150, 175 and 200 g/ha, Imidacloprid 200 SL @ 75, 100 and 125 ml/ha, Thiamethoxam 25 WG @ 75, 100 and 125 g/ha and Acetamiprid 20 SP @ 37.5, 50 and 62.5 g/ha were tested for their efficacy. Population of aphids per inflorescence before spray varied from 12.30 to 16.97. On third and seventh days after spray (DAS) Flonicamid @ 175 and 200 g/ha, Imidacloprid @ 100 and 125 ml/ha, Thiamethoxam @ 100 and 125 g/ha, Acetamiprid @ 62.5 g/ha were statistically better in reducing aphid population than other treatments. On tenth DAS 100 per cent mortality was observed in Flonicamid @ 200 g/ha, Imidacloprid @ 100 and 125 ml/ha, Thiamethoxam @ 100 and 125 g/ha and Acetamiprid @ 62.5 g/ha and these were statistically at par with Acetamiprid @ 50 g/ha and Flonicamid @ 175 g/ha. In standard checks Malathion 50 EC @ 1000 ml/ha only 55.50 per cent mortality was observed. Highest population of natural enemies mainly ladybird beetle was recorded in the plots sprayed with Flonicamid. Highest seed yield of 12.7 q/ha was obtained with Flonicamid @ 200 g/ha which was statistically at par with Imidacloprid @ 100 and 125 ml/ha, Thiamethoxam @ 100 and 125 g/ha and Acetamiprid @ 62.5 g/ha.

Keywords:*aphid, Celery, Myzus persicae, safer insecticides*

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Coadministration of wax dissolving agents with insecticides: An effective toxicity enhancing approach for the chemical control of adult female and ovisac stages of *Phenacoccus solenopsis*

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Adult female and ovisac stages of Phenacoccus solenopsis are very difficult to manage with synthetic insecticides because these stage life stages are protected by either thick waxy covering (adult female) or by the fluffy ovisac (ovisac stage). The biofactors of insect life help the pest ovoid the insecticide exposure and develop overlapping generations inspite of chemical application. The toxicity enhancing approach evaluated in the laboratory during present studies implied the coadministration of various wax dissolving agents with such insecticides which proved ineffective alone against adult female and ovisac stages of P. solenopsis. Wax dissolving agents in different combinations were evaluated in the laboratory alone or in coadministration with insecticides by topical application method. After topical application, ten specimens of respective treated stage of P. solenopsis were released inside the sterilized petridish having untreated pieces of pumpkin bottom part and sieved hole in the lid part of petridish which were then put inside the incubator maintained at 30±1 ºC and 65±5% rh. This approach synergized the toxicity of insecticide many fold enhancing the mortality of adult stages by many fold removal of wax and accelerated dehydration from the body as well as of ovisac stage by dissolving the fluffy ovisac and exposing the ovisac stage to insecticide contact. This approach would be a turning point in the chemical management of P. solenopsis. The details of the rest of results are discussed in the present manuscript.

Keywords:*Insecticide toxicity, Synergism, P. solenopsis, Laboratory bioassay, Wax dissolving agents*

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Pyrifluquinazon: A novel insecticide for managing *Bemisia tabaci* and transmission of *Tomato Yellow Leaf Curl Virus*

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Pyrifluquinazon a novel insecticide developed by Nihon Nohyaku Co., Ltd, Japan is used to control aphids, thrips, mealybugs and scale. Glasshouse studies were conducted at Department of Entomology, University of Georgia, Tifton to determine the efficacy of pyrifluquinazon in controlling Bemisia tabaci and in preventing acquisition and transmission of the begomovirus Tomato yellow leaf curl virus (TYLCV). Regarding settling behaviour of B. tabaci in pesticide treated plants, newer molecules like pyrifluquinazon and cyazypyr harboured minimum number of whiteflies. Efficacy of pyrifluquinapon on acquisition of TYLCV by B. tabaci showed that whiteflies caged in all the treated infected plants acquired the virus and were statistically on par in their acquisition rate. But, the whiteflies that were positive to TYLCV by PCR, died after feeding on the treated plants. Among the pesticide treated plants, viruliferous whiteflies fed on imidacloprid treated healthy plants could able to transmit TYLCV upto 10%. While, viruliferous whiteflies fed on pyrifluquinazon, cyazypyr and pymetrozine treated healthy plants could not be able to transmit TYLCV. Studies on efficacy of newer pesticide molecules on primary spread of TYLCV registered that among the treatments, viruliferous whiteflies fed on imidacloprid treated plants transmitted 31.9 per cent TYLCV infection followed by pymetrozine (12.5%), pyrifluquinazon (6.3%) and cyazypyr (6.3%). Whiteflies fed on untreated control resulted in 100 per cent TYLCV infection. These data justify inclusion of this novel insecticide with a new mode of action for foliar application in TYLCV management program.

Keywords:*Pyrifluquinazon, TYLCV, Bemisia tabaci*

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To determine the yield losses, caused by aphids in canola *Brevicoryne brassicae, Myzus persicae, Lipaphis erysimi* under field conditions in organic and inorganic systems of farming.

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Canola brassica napus is subject to attacked by various invertebrate pest all over the world (Lamb 1989). At least 30 species of invertebrates' pest have been found feeding on canola plants.There are three major aphid species infesting the canola crop, those are cabbage aphid, Brevicoryne brassicae (L.), the turnip aphid, Lipaphis erysimi Kalt, and the green peach aphid, Myzus persicae (Sulzer) (Hemiptera: Aphidae).Normally an aphid causes 30-35% yield loss on brassica plant, but if it is not controlled yield loss may rise up to 70%. The study was carried out in the farm area of university of agriculture, Faisalabad to determine the yield losses caused by aphids in canola crop. Canola crop was grown by organic and inorganic farmings. Seeds were sown with hand drill in a plot having three rows, with row to row distance 0.5m and plot to plot 1.5m. After germination cultural practices were performed through out the growing season uniformly in all plots. Aphids were allowed to develop on two rows, where as one row was kept free from aphids by spraying insecticides advantage in each treatment. During the application of insecticides, it was make sure that insecticides may not affect the aphids present on plants of remaining rows. There were negligible aphids on treated lines. Plants were inspected regularly for aphid population in all plots.

Keywords:*Canola aphids, yield loss, organic farming, inorganic farmings*

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Bio-Activity of *Secamone afzelii* (Schult) extracts for the management of beetles on stored cowpea seeds

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Insect damage of stored grains and pulses may amount to 10-40% in countries where modern technologies have not been introduced. The efficacy of oil extracted from leaves of Secamone afzelii was evaluated in the laboratory against Callosobruchus maculatus attacking stored cowpea. Leave extracts from Secamone afzelii was obtained by soxhlet extraction method using ethanol and hexane as the solvent. Each of the extracts was tested by exposing five pairs adult beetles to various levels of 0.5, 1.0, 1.5 and 2.0ml corresponding to 2.5, 5.0, 7.5 and 10.0% v/w admixed with 20g cowpea in three replications respectively in a Completely Randomized Design (CRD). Control treatment was set along. The result indicated that all the leaf extracts treatments recorded significantly high ($P<0.05$) adult mortality at 48 and 72 hours after treatment. oviposition of the female beetles was significantly reduced on seed treated with higher treatment level of extracts, in comparison with control. The extracts reduced the F1 progeny emergence, adult exit hole and percentage weight loss. However, among leaf extracts, ethanol leaf extract at 2ml (10.0% v/w)/20g cowpea seeds was most effective in reducing female oviposition and seed damage. Therefore, the extracts has great potential for use as a plant-based biopesticide for controlling pulse beetle C. maculatus.

Keywords:*biopesticide, leaf extract, oviposition, plant-based, pulse beetle*

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Prey suitability, population growth and development of*Orius maxidentex* Ghauri (Hemiptera: Anthocoridae)

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Laboratory studies on the biological information of the anthocorid predator, Orius maxidentex Ghauri was conducted. Firstly, prey preferences were done in choice and non-choice tests on four species of prey including Thrips palmi Karny, Aphis gossypii Glover, Bemisia tabaci Gennadius, and Tetranychus truncates Ehara. Both nymphs and adults of O. maxidentex preferred T. palmi to the other three species. In addition, 1st and 2nd instar nymphs of the predator did not consume A. gosypii. Subsequently, the life-table parameters of O. maxidentex were obtained only in T. palmi and B tabaci; because no nymphs survived to adults when fed with A. gossypii, and T. truncates. The net reproductive rate of increase (Ro, /) was higher when provided the predator with T. palmi (2.6200) than with B tabaci (0.6850); and the cohort generation time (Tc, day) 17.4200 and 16.5180; the capacity of increase (rc, / /day) 0.0553 and -0.0229; and the finite rate of increase (λ, / /day) 1.0568 and 0.9773, respectively. Finally, the life cycle of O. maxidentex fed with P. palmi revealed the incubation period of egg 3.26+0.44 days; the total nymphal development duration, 1st - 5th instars, 13.79+1.26 days. The means longevity of adult male and female were 7.95+1.39 and 9.31+2.51 days, respectively and with a sex ratio : 1:1.39. Hence, T. palmi was the suitable prey among the test species for mass production of O. maxidentex. A total number of 30,000 adults/month of the predator were mass-produced.

Keywords:*Orius maxidentex, prey suitability, biological information, life table*

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O407

Biological control strategies against Lepidopteran pests by using egg parasitoids

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Development of biological control strategies by using egg parasitoids has been performed for many years at INRA in the South of France. Each biological control program needed to be studied through some key steps, as following: 1.Pest studies and damage 2.Study of beneficial insects 3.Screening of parasitoid efficacy in laboratory, mesocosm,... 4.Release strategies of parasitoids 5.Optimization of field releases in crops 6.Optimization of mass production 7.Checking of field efficacy 8.Agro-system management and ecological effects Step by step, important programs on biological control against Lepidopteran pests by inundative releases of Trichogramma spp. were performed in our laboratory, thanks to large collaboration, against: ?Paysandisia archon, castniid palm borer (2012-2013), invasive pest in Mediterranean Bassin and Europe ?Tuta absoluta, tomato leafminer by studying about 100 strains of Trichogramma (2011-2013). Parasitoid efficacy in laboratory, mesocosme, greenhouse or in field has been evaluated, then compared to the control strain of Trichogramma achaeae, species currently marketed. ?Chilo sacchariphagus, stemborer of sugarcane : IPM strategy reduced up to 50% of damage with financial gains ranging from 800 to 1800 dollars per ha (associating inundative release of an egg parasitoid, Trichogramma chilonis and conservation of a predatory ant, Pheidole megacephala) (since 2000) ?Cacyreus marshalli, geranium bronze butterfly by studying 8 species of Trichogramma spp. (2000-2001) ?Plutella xylostella, diamondback moth (on cabbage) by using Trichogramma spp. (1998-2000) Sharing experiences and advances obtained these diverse research programs seems interesting for other projects by biological control and for the scientific community.

Keywords:*Trichogramma spp., cold storage, inundative release, Paysandisia archon, Tuta absoluta, Chilo sacchariphagus, Cacyreus marshalli, Plutella xylostella*

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O407F03

IPM

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Biological control against the tomato leafminer, *Tuta absoluta*, by studying egg parasitoids to improve IPM strategies

Hong Do Thi Khanh[1], Marion Tiradon[2], Anais Chailleux[3], Nicolas Desneux[4], Etty Colombel[5], Gilles Ridray[6], Frederic Rey[7], Anais Vernillet[8], Julien Seguret[9], Pascal Maignet[10], Yannie Trottin - Caudal[11], Catherine Chabriere[12], Jerome Lambion[13], Anne Terrentroy[14], Elisabeth Tabone[15]

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Since 2011, new egg parasitoids have been studied in our research program improving IPM strategies against Tuta absoluta, the South American tomato leafminer (TutaPI program, French funding, 2011-2013). This invasive pest in Mediterranean Basin and Europe may cause serious damage on tomato crops without efficient control methods. Greenhouse tests in IPM strategies combining inundative releases of Trichogramma achaeae (a commercially-available species) and the predator, Macrolophus pygmaeus, were efficient. This predator is also used against whiteflies on protected tomato crops. Other species of Trichogramma (19) from the whole world were investigated. Firstly, screening of parasitoid efficacy was performed in the laboratory for sixty-four strains (long-term rearing in the laboratory in the collection at INRA Provence-Alpes Cote d'Azur, France). Then, thirty strains collected last summer (from tomato crops in the South of France, collections performed by TutaPI partners) were studied. In laboratory conditions, some strains from both long-term rearing and field collections showed better efficacy than the control strain Trichogramma achaeae. The parasitoid efficacy is being studied in mesocosm, then in greenhouse to select the most efficient strains for biological control and IPM strategies. This work combined with other actions within the TutaPI program, aims to develop IPM strategies that are more efficient and at an affordable cost to growers.

Keywords:*Trichogramma spp., inundative release, invasion, tomato, invasive pest, greenhouse*

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Interspecific competition of parasitoids of *Bactreara dorsalis*(Hendel)

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Oriental fruit fly, Bactrocera dorsalis (Hendel) , is one of the most serious pests in China. It damages more than 250 species of fruits and vegetables belong to 40 families. Fopius arisanus (Sonan), Psyttalia incisi (Silvestri), Fopius vandenboschi (Fullaway) and Diachasmimorpha longicaudata (Ashmead) are the famous parasitoids of B.dorsalis in the world and they can attack eggs and different instars of the host. In order to control B. dorsalis effectively by parasitoids, the interspecific competitions of the 4 parasitoids were studied under laboratory conditions. The results showed that the larvae parasitoids P. incise and D. longicaudata could not discriminate the hosts which had been parasitized at egg stage by egg parasitoid F. arisanus but F. vandenboschi could. The D. longicaudata could discriminate the hosts which had been parasitized at young larva stage by P. incisi but not by F. vandenboschi. P. incisi and F. vandenboschi could not discriminate each other, but the success depended on the first.

Keywords:*Bactrocera dorsalis (Hendel) , parasitoid, interspecific competition*

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Investigations into diploid males in the *Wolbachia*-infected parasitoid, *Trichogramma kaykai* Pinto & Stouthamer (Hymenoptera: Trichogrammatidae)

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Sex determination in the haplodiploid order, Hymenoptera, has been studied extensively but is still not well understood. While cytoplasmic and environmental factors have been shown to influence sex determination in the Hymenoptera, the genetic mechanism, Complementary Sex Determination (CSD), is the only well understood mechanism of sex determination in this order. However, CSD does not explain sex determination in members of the superfamily, Chalcidoidea. In the chacidoids, temperature and cytoplasmic reproductive parasites, such as Wolbachia, may significantly affect sex determination. Female Trichogramma parasitoids infected with parthenogenesis-inducing (PI) Wolbachia typically produce exclusively diploid homozygous female offspring. However, these wasps can produce a small number of intersex and male offspring when exposed to high temperatures during late larval/ early pupal development. We examined 142 males produced by Wolbachia infected Trichogramma kaykai females using flow cytometric analysis and determined approximately 6% percent of were diploid. Further examination into the reproductive function of parthenogenetically produced diploid males showed that diploid males experience significantly reduced reproductive success than their haploid counterparts. However some diploid males are at least partially fertile and sire triploid females, presumably with unreduced sperm. These findings may provide valuable insight into the mechanisms governing chalcidoid sex determination as well as the methods by which reproductive parasites are able to manipulate sex in this group.

Keywords:*Chalcidoid, Complementary Sex Determination, sex determination, Wolbachia, triploid female*

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Sugar as nutritional supplement for the zoophytophagous predator *Nesidiocoris tenuis*: Practical implications

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Nesidiocoris tenuis (Reuter) (Hemiptera: Miridae) is a zoophytophagous predator widely used in integrated pest management programs in both, greenhouse and open-field, tomato crops in the Mediterranean basin. Its use, both by inoculation or conservation, may cause some drawbacks. First, to establish N. tenuis, inoculative releases are usually combined with the addition of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) eggs. The use of this factitious prey increases the cost of the release. Second, N. tenuis can cause some plant damage due to its phytophagous diet when prey is scarce. The aims of this work were: (i) to evaluate the effect of sugar addition on the biology and prey consumption of N. tenuis under laboratory conditions; (ii) to test whether the quantity of E. kuehniella eggs added as factitious prey could be reduced when sugar sources were also added under greenhouse and open-field conditions; (iii) to test whether the addition of sugar reduce the damages inflected by N. tenuis. Our results showed that the addition of sugar in an E. kuehniella-based diet increased N. tenuis progeny and reduced the predation rate of E. kuehniella eggs. In the field, the addition of sugars reduced the number E. kuehniella eggs necessary to establish N. tenuis and reduced the damage produced by N. tenuis. These findings may have practical implications, both in N. tenuis mass-rearing systems and in biological control programs where N. tenuis is released.

Keywords:*Miridae, sucrose, tomato, plant damage, mass rearing, inoculative releases, establishment, conservation*

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O407F07

IPM

O407

Comparative study of suitable host plants for adult oviposition and hatching ability of the predatory bug, *Wollastoniella rotundai* (Hemiptera: Anthocoridae)

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Wollastoniella rotunda Yasunaga & Miyamoto (Hemiptera: Anthocoridae) is an important natural enemies of tiny insect pests, especially thrips, Thrips palmi Karny. Mass rearing of natural enemies is crucial for biocontrol agents release. The objective of the study was to investigate the suitable host plants for the predator to lay most eggs and their hatching ability. The experiment was assigned in a completely randomized design with 20 replications. Seven species of plants were tested; they were two-month old eggplant plant, one-month old eggplant, sprouts of mungbean, blackbean, soybean, red kidney bean and peanut. Adults of W. rotunda did not survive when reared on sprouts of soy bean, red kidney bean and peanut. But they survived and reproduced on eggplant plants (both one- and two-month old), sprouts of mungbean and black bean. Number of eggs laid/plant/day were not statistically different ($P>0.05$) among two-month old eggplant plant (4.05±2.35), one-month old eggplant (5.05±3.71) and black bean sprout (4.30±3.10) but they were statistically different ($P<0.05$) from mungbean sprout (2.55±1.47). Furthermore, hatching percentage of eggs on two-month old eggplant plant (90.00±30.78) was statistically different ($P<0.05$) from one-month old eggplant (45.52±35.39), mungbean sprout (44.67±40.79) and black bean sprout (44.87±35.50). Consequently, two-month old eggplant was the most suitable host plants among the test plants, for initiating new culture of the predators.

Keywords:*Host plants, oviposition, Hatching ability, Wollastoniella rotunda*

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O407F08

IPM

O407

Effects of temperatures on shelf life of cocoon and adult survival for transportation of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae)

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Infestations of the lepidopteran sugarcane borer complex, Sesamia inferens (Walker), Chilo tumidicostalis Hampson, Chilo sacchariphagus (Bojer) and Chilo infuscatellus (Snellen), the most important pest insects of Thailand sugarcane plantations, occur annually. Two consecutive field experiments were performed with Cotesia flavipes (Cameron), a braconid biocontrol agent of the pest, in Central Thailand, with two objectives: to optimize rate and timing of field release of C. flavipes in order to suppress the pest infestations and to maximize the yield of sugarcane. Demonstration sites, in each experiment, were done in four field locations, two acres at each site, and in completely randomized block design. Results in the first experiment came out with the higher rate of release, the higher percent parasitisation obtained. With 375 and 500 adults release/acre yielded 22.48±4.03 and 25.12±3.77% parasitisation which were significantly higher ($P<0.05$) than those release with 125, 250 adults/acre and in control treatment (non-release), 14.81±4.11, 17.16±3.37 and 2.56±1.47, respectively. However, percent parasitisation in treatments, 375 and 500 adults/acre, were not significantly different ($P>0.05$). Later experiment, timing of release, with 375 adults/acre, appeared that monthly release of the parasites in 1-month old sugarcane sprouts gave 25.46±2.13% parasitisation and the sugarcane yield was 38.38 tons/acre which were significantly higher ($P<0.05$) than those in treatments, monthly release in 4-month old sugarcane; monthly release in 4-month old plus cut-off the insect-damaged shoots of sugarcane; and control (non-release). Percent parasitisation in the four later treatments were 21.42±4.31, 25.46±2.13, 21.48±2.00, and 6.20±0.91; and yields gained were 13.55±0.83, 14.68±0.37, 13.48±0.87 and 10.63±1.22 tons/hectare, respectively.

Keywords:*shelf life, Survival, Transportation, Temperature, Cotesia flavipes*

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O407F09

IPM

O407

Influence of abiotic factors on the parasitism rate of *Platygaster diplosisae*, Risbec and *Aprostocetus procerae*, Risbec on African rice gall midge (*Orseolia oryzivora*, Harris & Gagne)

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African rice gall midge (AfRGM), Orseolia oryzivora is one of the most damaging insect pests of lowland rice in Nigeria in the recent time, and could result in total crop failure in endemic areas. Of all the control measures adopted so far biological control is the most promising. Two parasitoids (Platygaster diplosisae, Hym: Platygastridae and Aprostocetus procerae, Hym: Eulophidae) have been identified with the potential to suppressed AfRGM population. In the current study, we evaluated the effect of abiotic factors (rainfall, relative humidity and temperature) in two eco-sites for two years on the the efficiency of the two parasotioids in the management of AfRGM. Percentage parasitisms by the two parasitoids were found to have significant correlation with the abiotic factors. Heavy rains and high humidity significantly reduced the efficiency of the two parasitoids. On the contrast, the population and efficiency of the parasitoids were observed to increase with reducing rainfall, RH, but increasing temperature thus increasing the efficiency of the parasitoids later in the season with up to 70% parasitism when combined, with P. diplosisae dominating the field. The results suggest that abiotic factors played significant role on the efficiency of these parasitoids and should be considered in the adoption of these parasitoids as bio-control agents of AfRGM.

Keywords:*Orseolia oryzivora, Platygaster diplosisae, Aprostocetus procerae, abiotic factors*

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O407F10

IPM

O407

Evaluating the added-value provided by genetic characterization methods in the classical biological control program against *Pseudococcus comstocki* (Hemiptera: Pseudococcidae) in France

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A classical biological control program against Pseudococcus comstocki (Hemiptera: Pseudococcidae) has been started in France in 2008. In this program, we have guided each step with genetic characterisation methods. First, we used DNA-sequencing to compare P. comstocki populations worldwide and identify their natural enemies. Second, we set up rapid PCR-based identification tools to support field surveys. Third, we developed microsatellite markers for the biocontrol agents Acerophagus malinus (Hymenoptera: Encyrtidae) and Allotropa burrelli (Hymenoptera: Platygastridae) and currently use them to characterise the populations to be field-released. This program is a good opportunity to evaluate the actual interests of using genetic methods. Indeed, although widely advocated, their use in routine is challenging: they must be set and used fast enough to provide relevant information when decisions are needed and must be at least as cost-efficient as other methods. Overall, the methods tested proved useful. DNA-sequencing avoided misidentification of P. comstocki with closely related taxa in several collection sites and allowed fast recognition of natural enemies without systematically sending specimens to taxonomists. PCR identification tools proved helpful to select future release sites because they allowed easy identification of field-collected mealybugs (as several species are often found in same agrosystems). Finally, although microsatellites are still under evaluation, they already successfully revealed loss of genetic diversity or detected between-populations contaminations in rearings. However, we still do not have strong evidence of a positive relationship between genetic diversity at microsatellite markers and performances displayed by the biocontrol agent populations.

Keywords:*mealybug, encyrtidae, SSR, DNA barcoding, acclimatation, species-specific PCR*

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O407

New host record for the egg parasitoid *Anagrus atomus* (Hymenoptera: Mymaridae) of the corn leafhoppers, *Z. sohrab, E. decipiens, A. decedens, P. striatus* (Hemiptera: Cicadellidae) and its population

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This study was carried out in order to determine the egg parasitoids of the four leafhopper species, which are the most abundant and common, at main and second crop maize cultivated areas in the South East Anatolia Region in 2009. Also the parazitoid was monitored its population fluctuation both main and second crop maize field in Diyarbakir province in 2010-2011. To obtain the egg parasitoids of the leafhoppers was used three different methods at laboratory and natural condition. Leaf samples infested with the leafhopper eggs were collected to observe its population fluctuation every week from the maize fields during vegetation. At the end of this study, only one dominant parazitoid species, Anagrus atomus (Linnaeus) (Hym.: Mymaridae), was determined. This is the first reference and record of this parasitoid reared from the eggs (Z.sohrab, E.decipiens, A.decedens and P.striatus) of the corn leafhoppers in Turkey. The parasitization rates were not measured because of rotting or dessication of some leaves containing exposed eggs. The population of A. atomus began to rise at beginning 2-4 leaf stage and reached to highest level at the maturation stage with parallel of the pests population development at main crop maize (on August) in both two years. Similarly results have been observed at second crop maize; the population of A.atomus has reached to highest level at flowering stage (on August).

Keywords:*Anagrus atomus, egg parasitoid, Cicadelliade, Leafhopper, Maize*

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O407F12

IPM

O407

Choosing predators for biocontrol of Colorado potato beetle in the South Ural

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Invasive species are the most problem in ecology. Trophic relationships of invasive species are very important for designing methods of pest management and biocontrol. We are researching the adaptation processes in predators proceeding to feed by Colorado potato beetle (Leptinotarsa decemlineata Say) in the South Urals. This species is invasive. It has appeared in South Urals 34 years ago. Complex of predators living in potato field is formed since its arrival Predatory insects were caught in antifreeze (water solution of ethylenglicol) and were preserved for a week. We extracted DNA from gut and its content; also we extracted DNA from other tissues (muscles, fat body). These samples of DNA were used for PCR with specific primers designed from COI of L. decemlineata. Predation in the insects can be detected by PCR analysis with species-specific primers even through 24 hours. Several species of Carabidae and Coccinellidae were caught. Seven-spotted lady beetle (Coccinella septempunctata L.), Pterostichus melanarius Ill., Harpalus rufipes De Geer and Calosoma investigator Ill. are most abundant predators in potato fields. The most positive results of PCR - analysis of predators gut content have been given in the case of C. septempunctata. Investigation funded RFBR (grant No 11-04-97022-r_povolzhie_a).

Keywords:*generalist predators, PCR analysis of gut content, Coccinella septempunctata, Leptinotarsa decemlineata*

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O407F13

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O407

Introduction of *Anagyrus lopezi* for biological control of the pink cassava mealybug, *Phenacoccus manihoti*, in Thailand

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The Pink cassava mealybug, Phenacoccus manihoti was first reported in Thailand in 2008 and by 2009 more than 230,000 hectares were infested. Chemical controls were attempted but could not contain the pest. Intensive surveys found no effective natural enemies. In West Africa, Anagyrus lopezi was reported as the most effective biological control agent of this mealybug. Five hundred adults of A. lopezi were introduced from International Institute of Tropical Agriculture, Benin by the Thai Department of Agriculture in September 2009. Host specificity testing showed the parasitoid attacked only P. manihoti and was safe for use as biological control agent in Thailand. Procedures for mass rearing of the parasitoids were developed and used mealybugs reared on cassava plants. Later rearing of mealybugs was on pumpkins which reduced the cost of parasitoid production. After obtaining a Release Permit, field releases started in July, 2010. 300 pairs of A. lopezi wasps per hectare were recommended for release on mealybug-infested cassava, but where heavy infestations occurred, 1250 pairs per hectare were recommended. Training courses were organized for transferring technologies to DOA researchers, extension officers and technicians of private sector. A total of 44 parasitoid rearing units were established at government and private sector facilities. Through this cooperative effort, more than 15,600,000 pairs of A. lopezi were released during 2010-March 2012 and as a result the outbreak areas were reduced to 4,190 hectares or 8.9% of total growing area, thus helping the cassava farmers and saving the Thai tapioca industry.

Keywords:*Pink cassava mealybug, Phenacoccus manihoti, Anagyrus lopezi, Introduction of parasitoid, biological*

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O407F14

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O407

Biological control of the millet head miner *Heliocheilus albipunctella* De Joannis (Lepidoptera: Noctuidae) by augmentative releases of the parasitoid wasp *Habrobracon hebetor* Say (Hymenoptera: Braconi)

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The pearl millet, (Pennisetum glaucum) production one of the most important staple crop in the Sahelian region is threaten by damages of insect pest. The millet head miner (MHM) Heliocheilus albipunctella (de Joannis) (Lepidoptera: Noctuidae), is one of the most important insect pests. Its developing larvae feed on the panicle and prevent grain formation. Among management strategies, biological control is one of the most promising methods. A biological control program was developed using an endogenous larval ecto-parasitoid wasp Habrobracon hebetor Say. The program was experimented in Burkina Faso, Mali and Niger from 2006 to 2009 by augmentative releases of the parasitoid. Parasitoids were allowed to reproduce and multiply in jute bags from which their offspring were able to escape through the jute meshes and dispersed to parasitize the MHM larvae in millet field. Our results indicate that up to 80% of H. albipunctella larvae were killed after releases of the parasitoids. As a result, damages of the MHM were reduced. This biological control program covered over 400 villages in the three countries and was positively perceived by farmers. Results are discussed in the perspective of sustainability and improvement of the program.

Keywords:*millet head miner, biological control, Habrobracon hebetor, farmer perceptions*

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O407F15

IPM

O407

Biological activities of emamectin benzoate and spinosad against *Trogoderma granarium*

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The present project was designed to evaluate the efficacy of spinosad and emamectin benzoate against Trogoderma granarium under the laboratory condition following completely randomized design. The experiment was carried out to check direct mortality and population build up. Patri dishes were used as exposure chambers. Concentrations of all the chemicals were prepared in acetone. 5, 10, and 15 ppm of Emamectin Benzoate and spinosad were used. Mortality data was recorded after 3, 5 and 7 days while post treatment build up of population was recorded after 35 and 70 days. Results showed that Emamectin benzoate showed more mortality than Spinosad and population build up was less in Emamectin benzoateas compared to spinosad

Keywords:*stored grain insects, spinosad*

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O407F16

IPM

O407

Biological characteristics, host stage preference and reproductive fitness of mealybug parasitoid *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae)

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Preliminary studies on biological characteristics, host stage preference by a solitary endoparasitoid Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae) and its effects on the reproductive fitness of A. bambawalei were studied on its natural host mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) reared on pumpkin in the laboratory at 28 ±2 oC, 65 ± 5 RH % relative humidity and 18 h light /6 h dark photoperiod. The mean development period of male and female parasitoid was 10.06 and 16.04 days, respectively. The mean development period between oviposition and mummy formation was 6.50 and 7.25 days for male and female, while from mummy formation to adult emergence it was 7.35 and 9.06 days for male and female, respectively. Females started oviposition on the day of emergence and males were short lived (18 days) as compared to females (40 days). A.bambawalei females parasitized 40-180 mealybugs during its life of 10-40 days and accepted mealybugs of all stages but preferred third instar nymphs and adults for oviposition. The sex ratio of resulting parasitoids was close to 1:2. The third instar mealybugs appeared to be the most appropriate hosts for the mass-rearing programme because they gave the best fitness return for the parasitoids. Parasitoid had an excellent searching ability and parasitized the mealy bugs in colonies and the scattered individuals on the plants. Keeping the heavy natural parasitization potential of the parasitoid in view, it is concluded that parasitoid must be conserved and further exploited for biological control of mealybugs.

Keywords:*Biological characteristics, Host stage preference, Sex ratio , Phenacoccus solenopsis, Aenasius bambawalei Hayat*

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O501TU01

Pathology & Microbial control

O501

Genome sequencing and structural characterization of a novel picorna-like virus in a laboratory culture of the endoparasitiod, *Pteromalus puparum* (Hymenoptera: Pteromalidae)

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Pteromalus puparum (Hymenoptera: Pteromalidae) is a dominantly pupal-stage parasitoid wasp of Pieris rapae. It has been verified that P. puparum/P. rapae system is a non-polydnavirus parasitization system, and venom is a key factor which provide protection for the parasitoid eggs against the host immune responses. However, here we reported on the presence of a novel picorna-like virus from P. puparum, designated as PpPRV, according to RNA sequencing and electron microscopy analysis of the parasitoid wasp. Depending on the transcriptomic data assembly and RT-PCR, we got the whole genomic sequence of this single-stranded RNA virus, the sequence represented that PpSRV belonged to picorna-like virus. The full length of the genome of was PpPRV was ~8.9 nt. Only one single open reading frame (ORF) has been found in the positive-sense RNA of PpPRV genome, which encoded two types of proteins. One type were non-structural proteins involving RNA-dependent RNA polymerase and helicase domains, and the other was structural polyprotein containing three conserved picorna-like domains, which were present in the amino and carboxyl-terminus, respectively. Characterization results showed that the genomic structure of PpPRV has high similarity with Marnavirus, another species of picorna-like virus. Although, according to the results of multiple sequences alignment and phylogenetic analysis, we inferred that PpPRV fell into the same clade of phylogenetic tree with a dicstrovirus named black queen cell virus, which contained two independent ORFs in its genome, encoded by positive-sense RNA. The novel discovery of PpPRV is new progress in the research of insect small RNA virus.

Keywords:*PpSRV, ORF, Marnavirus, Dicstrovirus*

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O501TU02

Pathology & Microbial control

O501

The MAP kinase BbSlt2 controls growth, conidiation, cell wall integrity, and virulence in the insect pathogenic fungus *Beauveria bassiana*

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Southwest University, China

Entomopathogenic fungi, such as Beauveria bassiana, are key environmental pathogens of insects that have been exploited for biological control of insect pests. Mitogen-activated protein (MAP) kinases play crucial roles in regulating fungal development, growth, and pathogenicity, mediating responses to the environment. BbSlt2, encoding for an Slt2 family MAPK, was isolated and characterized from B. bassiana. Gene disruption of BbSlt2 affected growth, caused a significant reduction in conidial production and viability, and increased sensitivity to Congo Red and fungal cell wall degrading enzymes. ΔBbSlt2 mutants were altered in cell wall structure and composition, which included temperature dependent chitin accumulation, reductions in conidial and hyphal hydrophobicity, and alterations in cell surface carbohydrate epitopes. The ΔBbSlt2 strain also showed hypersensitivity to heat shock and altered trehalose accumulation, which could only be partially attributed to changes in the expression of trehalase (ntl1). Insect bioassays revealed decreased virulence in the ΔBbSlt2 strain using both topical and intrahemoceol injection assays. These results indicate that BbSlt2 plays an important role in conidiation, viability, cell wall integrity and virulence in B. bassiana.

Keywords:*Beauveria bassiana, cell wall integrity, virulence, MAPK, entomopathogenic fungi*

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O501TU03

Pathology & Microbial control

O501

Baculoviruses - a useful tool for IPM strategies

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Baculoviruses are arthropod specific viruses that usually have narrow host ranges. There is a common consensus that baculoviruses are safe for the environment and human health. Baculoviruses have been predominantly considered to be valuable for biological control in organic farming. Recently the demand increased significantly to include biocontrol products such as baculoviruses also into IPM programmes. In Europe, Cydia pomonella granulovirus (CpGV) has broadly been accepted as a standard in IPM control strategies against codling moth in pomefruit. Far more than 100'000 hectare units of CpGV are applied annually. In Spain, the use of Spodoptera exigua NPV ensures effective control of beet armyworm, without harming beneficial insects. Such integrated control programmes help vegetable growers to produce at low residue levels, a prerequisite for marketing their products through European supermarkets. South African citrus growers successfully include Cryptophlebia leucotreta granulovirus in their control programmes against false codling moth, as export requirements demand low residues. Field trials in France showed that Helicoverpa armigera NPV can be a powerful tool for controlling bollworms in maize. Already one or two applications can effectively control the pest, and therefore the use of the virus offers an additional element for grower's plant protection strategies. The advantages of integrating baculoviruses into IPM strategies are (1) no residues, (2) no hazard for beneficial insects, (3) population control, and (4) resistance management. The key for a broader acceptance will not only be to offer products at competitive prices, but also to provide reliable instructions, appropriate logistics and consistent product quality.

Keywords:*baculoviruses, IPM, Cydia pomonella granulovirus, Cryptophlebia leucotreta granulovirus, Spodoptera exigua multiple nucleopolyhedrovirus, Helicoverpa armigera nucleopolyhedrovirus*

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O501TU04

Pathology & Microbial control

O501

Integrated pest management through sub-lethal synergy between chemical insecticides and entomopathogenic fungi

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Synergism between chemical insecticides and entomopathogenic fungi (epf) is well documented, an approach seeking to bridge the divide between chemical and biological pest suppression. The premise of this approach, being: two 'materials' assisting each other; the pathogen making the pest sick enough to become less resistant to the chemical and the chemical weakening the pest sufficiently to make it more susceptible to infection. Effective 'two-in-one' tank mixtures can reduce the quantity of insecticide needed (lower residues, costs) while at the same time address the risks of insecticide-induced resistance and environmental contamination. In South Africa, wheat (Triticum aestivum) is prone to infestation by any of six cereal aphid species, notably the Russian wheat aphid, Diuraphis noxia (Kurdjumov). The systematic development of host plant resistance-breaking biotypes of D. noxia implies that wheat producers may have to revert to using chemical insecticides with negative environmental consequences. Compatibility assays were conducted using two species of epf, Beauveria bassiana and Metarhizium anisopliae, with the insecticides Aphox® (pirimicarb), Mospilan® (acetamiprid) and/or Dursban® (chlorpyrifos), measuring percentage conidial germination and radial vegetative growth. Combinations tested were found to be compatible, except for B. bassiana + chlorpyrifos at concentrations ≥25% (of label rate). Comparatively, mortality of D. noxia following treatment with B. bassiana (mean 812±25 conidia/mm2), pirimicarb (1%) and combinations thereof, indicated an added benefit of ca.10% mortality (chemical + fungus), which may have accrued from chemically-stressed aphids being more susceptible to the fungal component in the mixture. Assays with additional combinations were subsequently performed and results are discussed.

Keywords:*Beauveria bassiana, pirimicarb, synergism, aphids*

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O501TU05

Pathology & Microbial control

Genetic evidence for a role of prophenoloxidase 1 in the *Drosophila* immune response

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Melanization is an invertebrate specific immune reaction that takes place within minutes following infection or wounding. During melanisation, phenols are oxidized into quinones that polymerize to form melanin. This reaction also leads to the production of reactive oxygen species that are thought to be directly toxic to microorganisms. Phenoloxidase (PO) is the key enzyme in this process. This enzyme is synthesized as an inactive zymogen called pro-phenoloxidase (PPO) that is activated by a serine protease cascade. Despite its conservation in many others invertebrates, melanization is one of the less characterized immune reactions in Drosophila. Moreover, some controversy exists on the relevance of the melanization reaction to combat pathogenic infections. To better understand the role of melanization in Drosophila immunity, we recently generated a null mutation in the gene encoding the Drosophila prophenoloxidase 1 (PPO1). To our knowledge, this is the first report of a PPO mutation among Insects. Interestingly, PPO1 mutant flies have a reduced phenoloxidase activity upon septic injury but a wild-type level of Toll and Imd pathway activity. However, PPO1 is required to combat infections with some Gram-positive bacterial species as PPO1 deficient flies show an enhanced susceptibility to Staphylococcus aureus. Surprisingly, PPO1 flies displayed an increased resistance to natural infection with Beauveria bassiana, an entomopathogenic fungus. Altogether our results indicate that PPO1 is an essential component of the antimicrobial response of Drosophila.

Keywords:*Drosophila, Immunity, Melanization, phenoloxidase*

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Pathology & Microbial control

O501

The damage efficiency of *Metarhizium anisopliae* and *Beauveria bassiana* on the larvae of coffee white stem borer *Xylotrechus quadripes* (Cherrolat) (Coleoptera: Cerambycidae) under laboratory conditions

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In Doi Chaang village, Chiang-rai Province, there is a major pest to Arabica coffee called the coffee white stem borer, Xylotrechus quadripes (Cherrolat) (Coleoptera: Cerambycidae). From January 2010 to December 2011, its reactions to Metarhizium anisopliae and Beauveria bassiana were tested under laboratory conditions. Through a completely randomized experimental design we used seven treatments of M. anisopliae and B. bassiana at various concentrations with 106, 107 and 108 spore/ml. and control (water mixed nonionic surfactant) were precisely evaluated. Five of the first insect instar larvae were infested by fungi and each treatment was replicated three times. The preliminary results showed that both fungus with the all concentrations treated at 108, 107 and 106 spore/ml could infect coffee white stem borer up to 100 percent after 4, 6 and 8 days after the infection respectively. M. anisopliae damaged larvae until dead with the appearance of white mycelium covered joints of larvae and then formed hold green spore all over the bodies. Meanwhile, B. bassiana showed only white. This investigation showed that both fungi have high potential to control coffee white stem borer.

Keywords:*Metarhizium anisopliae, Beauveria bassiana, Coffee white stem borer,*

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O501TU07

Pathology & Microbial control

O501

Various infection status and molecular evidence for horizontal transmission and recombination of *Wolbachia* and *cardinium* among rice planthoppers and related species

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Wolbachia and Cardinium are widely distributed and are considered important for their ability to disturb reproduction and affect other ?tness-related traits of their hosts. By using Multilocus Sequence Typing (MLST), RFLP (restriction fragment length polymorphism) and 16S rDNA gene sequencing methods, we extensively surveyed Wolbachia and Cardinium infection status of four predominant rice planthoppers and one kind of leafhopper in different rice fields. The results demonstrated that Sogatella furcifera (Horvath) and Laodelphax striatellus (Fallen) were infected with the same Wolbachia strain (wStri), while Nilaparvata lugens (Stal) and its closely related species Nilaparvata muiri China were infected with two phylogeneticlly distant strains, wLug and wMui, respectively. Three new Wolbachia strains (provisionally named wMfas1, wMfas2 and wMfas3) were detected in the leafhopper Macrosteles fascifrons (Stal). Only S. furcifera was co-infected with Cardinium, which indicated that the distribution of Cardinium in these rice planthoppers was narrower than that of Wolbachia. Unambiguous intragenic recombination events among these Wolbachia strains and incongruent phylogenetic relationships show that the connections between different Wolbachia strains and hosts were more complex than we expected. These results suggest that horizontal transmission and host associated specialization are two factors affecting Wolbachia and Cardinium infections among planthoppers and their related species.

Keywords:*Wolbachia, Cardinium, infection status, intragenic recombination, rice planthopper*

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O501TU08

Pathology & Microbial control

O501

Effects of *Maruca vitrata* multiple nucleopolyhedrovirus on non-target insect pests and natural enemies

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A nucleopolyhedrovirus (NPV) specifically infecting legume pod borer, Maruca vitrata, was characterized based on ultra-structural morphology, restriction endonuclease cleavage patterns, and sequences of the coding region of the polyhedrin gene, and named as MaviMNPV in Taiwan. This is the first report of a NPV infecting M. vitrata. Laboratory bioassays and field experiments had shown MaviMNPV is highly effective against early larval instars of M. vitrata. Because the newly identified MaviMNPV targeting M. vitrata may affect other insects, it is important to assess its activity against selected non-target insect pests and natural enemies that coexist with M. vitrata in vegetable legume production systems. MaviMNPV was tested at the recommended dose (106 OBs/ml) and a higher dose (107 OBs/ml) against non-target insect pests such as Asian corn borer (Ostrinia furnacalis), tomato fruit worm (Helicoverpa armigera), common armyworm (Spodoptera litura) and beet armyworm (S. exigua), and a generalist predator, green lacewing (Mallada basalis) that occur in vegetable legume production systems in Southeast Asia. MaviMNPV did not kill O. furnacalis, H. armigera, S. litura or S. exigua, nor did it affect larval mortality, larval period, pupal mortality, pupal period, pupal weight, adult emergence, fecundity and egg hatchability. MaviMNPV did not affect the larval survival, pupation and adult emergence of the predator M. basalis when it was fed with virus-infected M. vitrata larvae. We concluded that MaviMNPV did not have any adverse effects on non-target insect pests and predators that coexist with M. vitrata in vegetable legume production systems.

Keywords:*MaviMNPV, non-target insect pests, generalist predator*

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O501TU09

Pathology & Microbial control

O501

Localization of deformed wing virus (DWV) and Korean sacbrood virus (KSBV) in olfactory organs of honeybees

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The honeybee is an important economic resource insect for the pollination of many crops, and a unique model to study nervous system processes which shows complicated social behaviors. But viruses are one of the most major threats to the honey bee colony losses around the world and cause serious concerns for beekeepers and researchers. In vertebrates and invertebrates, virus infections affect morphological and functional changes in the nervous system including a wide range of sensory, motor function, and other behaviors. Deformed wing virus (DWV) is a positive-strand RNA virus and detected in many bee colonies, indicating that DWV infection is very abundant and lead to deformation of wings, paralysis and mortality of emerging bees. Honeybee sacbrood virus (SBV) belongs to picornavirus, a small positive strand RNA virus, causing the failure to pupate and death in both larval and adult bees. In this study, we investigated infectious pathways of two viruses into the haemolymph and identified the presence of DWV and SBV viral RNA in several areas involved in olfactory systems in honeybees.

Keywords:*Apis mellifera, Honeybee, DWV, KSBV, olfactory system*

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O501TU10

Pathology & Microbial control

O501

Biological control of *Plutella xylostella* L. and *Aedes aegypti* L. larvae by using *Bacillus thuringiensis* Fusants.

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Pathogenicity of five fusants of Bacillus thuringiensis namely F2, F4, F16, F21 and F36 to P. xylostella and Ae. aegypti larvae were investigated to find an alternative of bio-agent to control both insects. The method used for pathogenicity tests was dip leaf bioassay method, method, and LC50 and LC95 values of five Bt. fusants were analysed by using Probit analysis and Abbot formula to determine LC50 and LC95. The research showed that five strains of Bt. fusants showed specific pathogenic to larvae P. xylostella and Ae. Aegypti. Fusant F16 was the highest pathogen against P. xylostella with LC50 = 3,37 x 1016 cell/ml and LC95 = 2,36x1031 cell/ml at 48 hours after treatment but was not pathogen to Ae. aegypti larvae. Meanwhile, Fusant F36 has the highest pathogenicity to Ae. aegypti for both the second and third instar larvae with LC50 and LC95 values for the second instars larvae were 4,394x105 and 2,423x107 cell/ml at 48 and 72 hours respectively where as it was not pathogen to P. xylostella . the other three fusant strains were not pathogen to both insect. As a conclusion there were no fusants have a double pathogenic against P. xylostella neither Ae. Aegypti.

Keywords:*Pathogenicity, Bacillus thuringiensis, fusant, Plutella xylostella L., Aedes aegypti L.*

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O501TU11

Pathology & Microbial control

O501

White grub pathogens and characterization of scarab species in Mauritius and Tanzania

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The present paper focuses on a EU-funded ACP Sugar Research project on the development of biopesticides for the management of white grub species in sugar cane in the Southern African Development Community (SADC) region. The prevalence of indigenous entomopathogens on the scarab species in Mauritius and Tanzania is described. On-going research on pathogen detection revealed the presence of entomopathogenic bacteria, nematodes and fungal pathogens namely Metarhizium anisopliae, Beauveria sp. and Orphiocordyceps sp. on the white grub in Tanzania. Several isolates of the former species, bacteria and nematodes were also detected on white grubs in Mauritius. In addition, Beauveria sp. was identified from soil samples. Investigations into the species composition and population dynamics of white grubs using molecular diagnostic techniques are also described. Accurate species identification is essential to ecological studies and to formulate appropriate pest management strategies. Integrated morphological and molecular studies were necessary to demonstrate the grouping of pleurostict scarabs prevalent in both countries. Two mitochondrial (cox 1 and 16S rRNA) and one nuclear (28S rRNA) gene regions had been used. The phylogenetic tree obtained by the Neigbour Joining method showed high congruence indicating the reliability of the gene regions and primers used. Several species had been identified belonging to three subfamilies of scarabaeidae namely Dynastinae, Melolonthinae and Rutelinae. The results show that DNA barcoding can be used to complement or ascertain morphological identifications. Results from the present work will be used to establish the links between the pathogen and the host insect.

Keywords:*Metarhizium, Beauveria, Orphiocordyceps, nematodes, DNA, mitochondrial, cox 1, 16S rRNA, 28S rRNA*

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O501TU12

Pathology & Microbial control

O501

Effects of entomopathogenic fungal endophytes on mortality, oviposition, emergence and longevity of *Liriomyza huidobrensis* (Diptera: Agromyzidae)

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Liriomyza leafminer flies are exotic pests of economic importance on horticultural crops in Africa and are also treated as quarantine pests in overseas markets. Parasitoids and entomopathogens are among the best control options for this pest. Ten fungal isolates belonging to the genus Beauveria (3), Hypocrea (1), Gibberella (1), Metarhizium (2), Trichoderma (1) and Fusarium (2) were screened for their endophyte properties on Vicia faba and Phaseolus vulgaris. Beauveria (IC279), Hypocrea, Gibberella, Fusarium (M6SF1, M7SF3) and Trichoderma isolates colonized the total host plant of both species. Beauveria isolates G1LU3 and S4SU1 colonized the whole plant of P. vulgaris but only the root and stem in V. faba. Isolates of Metarhizium failed to colonize any of the two host plants. The effects of fungal endophytes on mortality, oviposition, emergence and longevity of L. huidobrensis were tested in planta through colonization of V. faba plants. All the fungal endophyte isolates that succeeded in colonizing the whole or parts of plants were pathogenic to L. huidobrensis causing 100% mortality within 13.2±0.7 and 15±0.6 days. However, the Hypocrea isolate outperformed the other isolates in reducing adult survival (3.8±1.0 days vs. 9.9±1.8 days in the control), number of pupae (80±6.7 vs. 387±21.7 pupae in the control), longevity of the progeny (11.2±1.0 vs. 17.8±1.4 days in fungus-treated and control, respectively) and adult emergences.

Keywords:*Endophytes, Beauveria, Hypocrea, Gibberella, Fusarium, Trichoderma, Pathogens.*

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O501TU13

Pathology & Microbial control

O501

Epidemiological studies on tick and mange infestation in camels in Pakistan (Cholistan, Bahawalpur).

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Prevalence of ecto-parasites in camels (Camelus dromedarius) was investigated in Cholistan, Bahawalpur-Pakistan. Ticks (Acari: Ixodidae) are one of the most prevalent ectoparasites in camels that cause acute or chronic illnesses, and can transmit a variety of disease agents to human beings, domesticated animals, and wildlife. Ticks have high reproductive potential, few natural parasites and predators, the ability to live several months or years, and relatively broad host ranges. They are well adapted for survival and difficult to control. In the present study clinical and epidemiological aspects tick and mange infestations of Cholistani camels in Bahawalpur region were reported. A total of 450 camels were examined from 90 herds, 250 animals were found to be infested with ticks and mange infestation from 70 different herds. The overall prevalence of ticks and Mange infestation was 55.55% and 40.80% respectively. The highest district wise prevalence was noted at Rahim Yar Khan (60.87%), followed by Bahawalnagar (49.75%) and the lowest at Bahawalpur (41.05%). The camel ticks on order of their preference were as: Rhipicephalus spp (28.95%) Hyalomma dromedarii (26.48%), Dermacentor sp (18.29%) H. anatolicum (12.47%), H. marginatum (6.69%), Ornithodoros spp. (4.89%) and Amblyomma variegatum (2.20%). Heavy infections lead to decrease in draught capacity, milk production in lactating dams and growth rate in young animals. Chrysomyia spp. and Wohlfahrtia magnifica are the most important myiasis producing flies affecting camel and cause vaginal and preputial myiasis. Cephalopina titilator fly was found to cause nasal myiasis in camel. The tick load per animal was higher during summer.

Keywords:*Ticks, Mange Infestation, Dipteran larvae, Wohlfahrtia magnifica, Camels, Cholistan, Pakistan.*

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O501TU14

Pathology & Microbial control

O501

Effect of Xennorhabdus nematophila on *Microplitis mediator* Haliday

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Xenorhabdus nematophila HB310(Xn HB310), an entomopathogenic bacterium appeared high toxic activity against several species of insect including Helicoverpa armigera (cotton bollworm, CBW). We investigated the effects of the culture broth and protein toxin of sublethal concentration on the development of the parasitic wasp of CBW larvae, Microplitis mediator. The results showed that 2.24×106 CFU/mL culture broth and 567.60 μg/mL protein toxin had not direct influence on the pupae emergence of M. mediator. When fed on the 10% honey with the culture broth(? 1.12×105 CFU/mL) or protein toxin (?200μg/mL), the longevities of M. mediator adults were shorten significantly. When the CBW larvae was fed on the diet containing the culture broth (1.12×103CFU/mL) from neonates on, there was no effects on the growth and development of M. mediator in the CBW larvae. Compared with the control, the larval duration of M. mediator was delayed significantly when the host larvae fed on the diet containing 0.38 μg/mL protein toxin from hatch. When CBW larvae fed on the diet with ?IC50 culture broth or protein toxin from parasitized 2nd larvae on, there was no detrimental effect on the development of M. mediator in CBW larvae. The wasp adult would not fed on high dose of Xn HB310 culture broth or protein toxin in natural conditions. These results supported the conclusion that the application of Xn HB310 is safe on the M. mediator.

Keywords:*Xenorhabdus nematophila, Helicoverpa armigera, Microplitis mediator, development*

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O501TU15

Pathology & Microbial control

Bark beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Curculionidae: Scolytinae) is a vector of mango

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Role of bark beetle Hypocryphalus mangiferae (Coleoptera: Curculionidae: Scolytinae) was assessed and proved as a definitive vector in the transmission of Mango Sudden Death Syndrome (MSDS). Studies were carried out in the southern Punjab, Pakistan. Healthy plants of mango (Mangifra indica L.) were enclosed with beetles with and without fungus inoculum by employing a technique "Mesh Enclosure Method" (MEM). Symptoms expression revealed that the wilting was significant in 3 and 6 months but no significant differences in oozing were found and also other symptoms like black streaks and cankers were observed in the plants on which infested beetles were introduced. Re-isolation of fungi after 7.5 months showed higher frequency of Lasiodiplodia theobromae compared to Ceratocystis fimbriata. In order to confirm the results, both of the fungi were also isolated from adult beetles and beetle's frass. Based on our findings, H. mangiferae was found to transmit the infection of Mango Sudden Death Disease in healthy plants.

Keywords:*HYPOCRYPHALUS MANGIFERAE, Diseases, Vector, Mango*

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O601M01

Medical Entomology O601

Entomological surveillance on a continental scale: A new application of social networking

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Arthropod surveillance is often limited by the short seasonal window of activity, funding, shortage of collection equipment, and the laborious nature of mosquito collection and species level identification; therefore simultaneous sampling of disease vectors on a continental scale has not been achieved in North America. By using social networking to contact and coordinate mosquito collections the Arthropod-Borne Animal Diseases Research Unit, a part of the United States Department of Agriculture, was able to collect 453 mosquito populations of Aedes vexans and Culex tarsalis throughout North America during the summer of 2011. Mosquito abatement districts, health departments, and individuals (530 total) were contacted by email (320), phone call (142), face-to-face (58), or by another method (10). These contacts demonstrated the value of social networks by recruiting an additional 202 people, a 38% increase. However, the importance of personal contact cannot be understated. Although "face-to-face" contacts represented only 8% of the total contacts, they provided 23% of the mosquito populations. Methods for finding, contacting, coordinating, and motivating contacts will be discussed in the presentation. Mosquitoes and the diseases they transmit are not limited by geo-political boundaries, therefore in the future social networking may increase coordination and effectiveness of mosquito abatement projects across the target species' entire geographic distribution.

Keywords:*Disease Vectors, Social Networks, Mosquito Collection*

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O601M02

Medical Entomology

O601

Mosquito monitoring program at Honolulu International Airport, Hawaii

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A mosquito surveillance program using Ovi-traps has been in continuous operation at the Honolulu International Airport for 2 years (May 2010 to April 2012). To the best of our knowledge, this is the first use of routine Ovi-trap monitoring at that site. Previous to this project, gravid and light traps were standardized components of public health mosquito surveillance at the airport. That effort was terminated in 2009. The current program focuses on (1) monitoring for the long-established day-time mosquito Aedes albopictus and (2) surveying for the introduction of mosquito species new to Oahu or the state. A. albopictus egg counts have fluctuated over time during the surveillance program. Preliminarily, we can say that oviposition at this site increases throughout the rainy season (October ? April). Most of this seasonal increase in mosquito oviposition occurs in the 2nd ? 3rd months of the rainy season, when heavy rains peak and then decline. Oviposition decreases within one month of consistent declines in rainfall at the end of the rainy season. Inter-annual variations within this generalized pattern are evident. In January 2012, A. aegypti was detected from a small garden located between terminals for out-of-state and within-state flights. This species had not been confirmed present on Oahu since 1949. This recent finding of A. aegypti has led us to intensify our surveillance program at the airport, with the hope that we will improve our understanding of the nature of mosquito introductions at this important port of entry for the Hawaiian islands.

Keywords:*Aedes albopictus, Aedes aegypti, invasive pests surveillance*

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O601M03

Medical Entomology

O601

Culex molestus and implications for mosquito-borne disease risk in Australia.

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The Culex pipiens subgroup of mosquitoes includes important species involved in mosquito-borne disease transmission internationally and four species within this subgroup are found in Australia. One of these species, Culex molestus, is thought to have been introduced into Australia in the 1940s but its importance as a vector of mosquito-borne viruses is not well understood and there is some debate regarding the relationship between this species and others in the Culex pipiens subgroup. Laboratory studies have shown that the species is uniquely adapted to subterranean habitats and the current trend towards increased water storage in urban areas has raised concerns of increased nuisance-biting and public health risks in the future. Concern has been raised as to the potential role this species may play in the transmission of exotic arboviruses such as West Nile virus and locally important arboviruses such as Murray Valley encephalitis virus. However, notwithstanding the vectorial capacity of this species, there may be ecological barriers that diminish the public health risks associated this species. This paper will present the findings of biological, ecological and molecular studies of Culex molestus with implications for the assessment of mosquito-borne disease risk.

Keywords:*mosquito, Culex pipiens, Culex molestus, subterranean habitats, molecular studies.*

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O601M04

Medical Entomology

O601

Aedes aegypti productivity and fecal contamination in domestic water storage containers in two villages in Northeastern Thailand

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Little is known about the relationship between Aedes aegypti production and fecal contamination in domestic water storage containers. To investigate this relationship, we conducted a cross sectional survey in NE Thailand in 122 and 128 houses in one rural and one suburban village, respectively. Overall, Ae. aegypti container, house and Breteau indices (18.9, 73.6 and 140) were above the WHO transmission thresholds for yellow fever, which are also applied to dengue, with a significantly higher container index in the suburban village ($p<0.05$). Overall pupae per person and pupae per house were 2.4 and 8.2 respectively, with no significant difference between villages. Seventy one percent of Ae. aegypti infested containers were positive for Escherichia coli, a commonly used indicator of fecal contamination and diarrheal disease risk, with the odds of E. coli contaminated containers being infested with Ae. aegypti ranging from 3.8 in the rural village to 5.7 in the suburban village. Ae. aegypti productivity was significantly higher in domestic containers co-infested with E. coli than in containers infested with only Ae. aegypti ($p<0.01$). Similarly, significantly higher levels of E. coli contamination were detected in containers infested with Ae. aegypti ($p<0.01$). The evident relationship between Ae. aegypti productivity and fecal contamination suggests that domestic containers in these settings are potentially important determinants of dengue and diarrheal disease. These containers could be excellent targets for combined control of both diseases. In further research, we will explore how this relationship can be targeted to develop integrated dengue and diarrheal disease control strategies.

Keywords:*Domestic water containers, Aedes aegypti, Thailand, Fecal contamination, E. coli*

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O601M05

Medical Entomology

O601

African Horse Sickness, its *Culicoides* (Diptera, Ceratopogonidae) vector and Hartmann's mountain zebra in the south-west Khomas Region, central Namibia.

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African Horse Sickness (AHS) is considered endemic to Namibia. Four of the nine known AHS virus serotypes (AHSV 1, 2, 4 and 9) were recently isolated from horses around the Windhoek area. Farmers have reported several horse casualties in the south-western Khomas Region, which was unexpected, as the area is particularly dry ? conditions thought unfavorable for the vector of African Horse Sickness Virus, namely Culicoides midges. The objective of this study was to determine the presence of factors necessary for the maintenance of AHS in the Khomas Region of Namibia, namely the Culicoides vector and a suspected African Horse Sickness Virus reservoir host or source, the Hartmann's mountain zebra. The distribution and abundance of Culicoides species was determined and an unexpected high diversity of species was found even during winter months. Hartmann's mountain zebras were found in significant numbers along the survey transect. ELISA tests of blood samples taken showed a 100% presence of AHSV antibodies in all the samples, indicating the survival of the zebras after exposure to the virus. This study indicated the importance of human settlement in the Khomas Region on the Culicoides distribution by investigating the background Culicoides populations away from homesteads. Further research is identified to determine the importance of the Khomas Region as an endemic source of African Horse Sickness Virus in the region.

Keywords:*African horse sickness*

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Medical Entomology

O601

Survey of some bionomic factors of *Culex tritaeniorhynchus* (Diptera: Culicidae) as vector of Japanese Encephalitis (Brain fever)

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Background: Diseases transmitted by arthropods are among the major causes of illness and death in many tropical and subtropical countries. Among these communicable diseases, Japanese encephalitis is endemic in at least 21 countries. Rapidly changing environment brings about frequent changes in vector behavior, which necessitate the regular general information on vector bionomics, so the present study undertaken to identify some ecological parameters that affect on vector of JE. Material and Methods: Mysore city of Karnataka state, India were selected for the present study mainly because of the endemicity of JE and prevalence of Cx. tritaeniorhynchus, the major vector of JE. The larvae of Cx. tritaeniorhynchus and associated species have been collected from temporary and semi permanent ground water pools. Simultaneously they have also been collected from plain rice field area from a 26 km radius outside Mysore. For the present study all the abiotic factors and one biotic factor estimated were subjected to statistical analysis to determine whether these could influence the larval density of Cx. tritaeniorhynchus. Results and Discussion: In the base of standardised coefficients Beta the most effective variables (predictor) on larval density among the 3 variables such as pH, height of paddy and density of Cx. fuscanus are height of paddy and pH. These results relating to some aspect of ecology of Cx. tritaeniorhynchus will help us to manage this species as well as assess the effects of human activities including land use pattern that could create epidemics of brain Fever and other mosquitoes borne diseases.

Keywords:*Japanese Encephalities. Culex tritaeniorhnchus. Ecolog, Bionomic*

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O601M07

Medical Entomology

O601

Survey of some epidemiological factors of visceral leishmaniasis transmitted by *Phlebotomus* sandflies (Diptera: psychodidae) in south of Iran.

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The leishmaniasis are estimated to affect 10-50 million people in the world. Visceral leishmaniasis (VI) caused by leishmania infantum, is endemic in several parts of world especially in Mediterranean regions. This disease is endemic in southern and north- west area in Iran. This disease without diagnosis and treatment can lead to death. We need epidemiological study including demographical factor, vectors, reservoirs and agents for the best way to control and prevention of this sandfly borne diseases. In this regard for the first phase of this investigation the files of 141 cases were studied with diagnosis of VI in Namazi hospital of Shiraz (Iran) during 2006-2011. Results showed that 54% of patients were boys and 46% were girls. The peak incidence of VI observed in winter (35.46%) followed by spring, summer and autumn respectively. Mortality was reported 9.20% in this study and 9.12% of patients developed relapse of disease. More patients were from rural areas. The species of Phlebotomus sandflies that were investigated as vectors of VI in Iran are: ph.major, ph. keshishiani, ph. kandelaki, ph. alexandri and ph. perfiliewi. The results of this research are useful for control of VI in this endemic area of disease in Iran.

Keywords:*Visceral Leishmaniasis, Phlebotomus, Epidemiology,*

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O601M08

Medical Entomology

O601

Goat hoof myiasis caused by *Wohlfahrtia magnifica* (Diptera: Sarcophagidae) larva in Dashte-arjan region, Fars, Iran

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Introduction and Objectives: Myiasis, is the infestation of tissues and organs of living vertebrate animals and humans by larval stages of Diptera. It is a world-wide problem, particularly, in tropical regions and a cause of severe financial losses. Although it is more prevalent in animals, it is also a relatively frequent occurrence in humans in rural areas. The main purpose of this study was to identify and characterize the flies responsible for goat hoof myiasis in Dashte-arjan region of Fars province in Iran. Materials and Methods: The Larvae were removed from the hooves, manually. Thereafter, they were identified by light microscopy on important morphological characteristics such as posterior spiracles and pictorial key. Results and Conclusions: Wohlfahrtia magnifica was isolated from goats' hooves. About 5% of the examined goats had larvae-infested wounds. Cases of myiasis were presented in July. The severity of infections ranged from 5 to 8 larvae in each wound. The case presented is the first case of Wohlfahrtia-induced myiasis in goat hoof in Fars province and indicates that the condition is endemic in the region.

Keywords:*Wohlfahrtia magnifica. Goat hoof, Myiasis. Fars. Iran*

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Medical Entomology

O601

Ecology of malaria vectors in southern part of Iran.

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Malaria is an unstable (stability index<0.5) and mesoendemic disease in Iran. National Malaria Control Program reported 13,821 malaria cases in 2004 of which 45% were imported. Subsequently, a total of 18875 cases were nationally reported in 2005. There appears to be no report on the epidemiological aspects of human malaria in the rapidly growing energy centre, Assalouyeh,. Assalouyeh petrochemical zone in the northern coast of the Persian Gulf is one of the main malaria foci of Iran. It is situated in the Indo- Persian subcontinental epidemiological zone of malaria (Onori et al, 1993), which is a very fast growing field of oil and natural gas. The large influx of international job seekers, as well as the internal immigrants to this centre (>30,000 annually), the presence of capable vectors, ecological instability due to the high rate of human movement and the fragile infrastructure of the local health system are among the main reasons of malaria expansion in recent years. Following an outbreak of malaria in 2002, this research was undertaken in Assalouyeh for the first time to find out the local prevailing circumstances, detect the eco- epidemiologic parameters and response rate of the main malaria vectors to the current control measures.

Keywords:*Malaria, vector, Iran, Anopheles, Plasmodium.*

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O602TH01

Medical Entomology

O602

Toxin peptide produced by *Xenorhabdus* species with a novel mode of action against yellow fever mosquito larvae, *Aedes aegypti*

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The goal of this study is to examine the toxicity and mode of action of a lipopeptide against larvae of Aedes aegypti, the yellow fever mosquito. Preliminary studies suggested that Xenorhabdus sp. produces small molecular weight peptides that are highly toxic yet specific to larvae of different mosquito species, including Aedes aegypti, Culex pipiens, and Anopheles gambiae. Further studies have been conducted to understand how the toxin peptides affect mosquito larvae. The results from this study indicated that the toxin peptide from Xenorhabdus sp. has acute toxicity against mosquito larvae at very low concentration with different mode of action from Bacillus thuringiensis israelensis toxin (Bti). Based on the cell assays, it was apparent that the toxin peptide is very specific toward mosquito cells, which indicated promising characteristics to be developed as next generation bio-pesticide.

Keywords:*Microbial Pesticide, Yellow Fever Mosquitoe, Aedes aegypti, A novel mode of action*

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O602TH02

Medical Entomology

O602

Potent repellents for control of savannah tsetse (*Glossina* sp.)

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Repellents for the control/management of disease vectors like tsetse have been identified at icipe from synthetic sources and from natural blends of un-preferred animals (e.g. waterbuck), which are common in tsetse habitats but not fed upon. The synthetic tsetse repellent 2-methoxy-4-methylphenol (icipe patent) and the 5-constituent waterbuck repellent blend (icipe patent) are being validated in large-scale field trials. A major constraint of the technology however is the dispensing of the repellents on the host. At the moment the repellent collar prototype, breaks easily and needs to be commercially produced. New dispensers (though still not optimal) have been developed and are currently being tested in field trials. The performance of the two repellents in field experiments and their potential in tsetse management will be highlighted. Despite the prototype nature of the technology, our results indicate that these repellents provide substantial protection to cattle either in 'push' mode or when used in conjunction with baited traps in 'push-pull' strategy. Most livestock keepers prefer the repellent technology compared to other tsetse and trypanosomosis control options due to the technology's simplicity and mobility. Investigations of feeding preferences of other vectors may similarly yield useful tools and tactics for their control.

Keywords:*repellents, tsetse flies, trypanosomiasis, repellent cow collars*

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O602TH03

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O602

The use of entomopathogenic fungi in the control of tsetse flies

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Tsetse flies are vectors of trypanosomes, which cause African trypanosomosis or sleeping sickness in humans and "nagana" in animals. About one-third of the continent, or nearly 9 million km2 is infested; thus, preventing full utilization of the best agricultural/grazing lands on the continent. Due to the lack of vaccines, inexpensive drugs and the development of resistance to drugs by the trypanosomes, vector control remains the only viable option for management of these diseases. The use of insect pathogens that occur naturally in insect populations represents one of the alternatives; but their potential as biological control agents has not been fully exploited, especially due to the difficulty of their application in the field because of the haematophagous behavior of tsetse flies. Since entomopathogenic fungi (EPF) infect their target organisms through the cuticle, as opposed to other pathogens that need to be ingested, it has been possible to develop a device that delivers and autodisseminate inoculum among tsetse in the field. The potential of this technique was tested in the field and results showed a reduction of tsetse population comparable to the conventional mass trapping technology. However, the success of this technology depends on the effective horizontal transmission of the inoculum between insects. We highlight the prospects of using EPF in combination with other technologies for the control of tsetse flies and the challenges related to the application of the technology.

Keywords:*Entomopathogenic fungi, tsetse flies, biological control*

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O602TH04

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O602

Cost efficient control of tsetse flies

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Tsetse flies (Diptera: Glossinidae) are the vectors of human and animal trypanosomiasis (also called Sleeping Sickness in humans and Nagana in animals). These diseases affect more than 36 countries in sub-Saharan Africa. Research and control of the disease has been ongoing since the early 1900's, yet trypanosomiasis is still responsible for economic losses estimated at US$4.5 billion per year, with a human DALY index of 1.59 million. Disease control is based on breaking the parasite lifecycle with drugs, or targeting the vector to interrupt transmission. For economic and sustainability reasons, vector control is a preferred option, but the ecology and biology of tsetse flies limits the techniques that can be used. For example, savannah tsetse species can successfully be eliminated from a 10 000km2 area for US$ 1million, with insecticide-treated targets. To achieve the same for riverine tsetse species (the major vectors of human trypanosomiasis) with standard methods, would cost US$ 7.5 million, due to dense habitat and poor response of riverine tsetse to current control devices. This presentation reports on a new, significantly cheaper insecticide-treated target for control of riverine tsetse flies. In an ongoing island trial against Glossina f. fuscipes, a population reduction of 99% was achieved within five months. Currently this technology is being evaluated in a large 500km2 trial in north-western Uganda, a foci for human trypanosomiasis. This is the first major advance in control of riverine tsetse species in almost three decades, and provides a crucial tool for management of this neglected tropical disease.

Keywords:*trypanosomiasis, tsetse flies, control.*

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O602TH05

Medical Entomology

O602

New tools for addressing insecticide resistance in malaria vectors

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The emerging and rapid spread of insecticide resistance threatens the utility of valuable interventions such as long lasting insecticidal nets (LLINs) and indoor spraying of residual insecticides. To inform the optimal deployment of existing insecticide-based tools as well as the development of the next generation of tools, an online interactive platform was created to map reports of insecticide resistance since the 1950s (www.irmapper.com). Data from WHO susceptibility tests are selected by mosquito species, insecticide type and mosquito collection period to produce tailored maps showing key information. Data on target site and metabolic resistance mechanisms are similarly displayed. Information was used to select sites in Uganda(4), Democratic Republic of Congo(3) and Ethiopia(4) for bioefficacy evaluations of WHO-recommended LLINs. Standard LLINs and a combination LLIN were assessed via cone bioassays using local pyrethroid-resistant Anopheles gambiae. Significant reductions in susceptibility to standard LLINs were observed across all 11 sites depending on net type, with minimum mean mortalities of 46-61% for Uganda, 41-52% for DRC and 15-34% for Ethiopia populations. Conversely, the combination LLIN performed well at sites in Uganda and DRC (mean mortality of ≥95%) with reduced efficacy observed for the insecticide-only sides but not the synergist plus insecticide roof for Ethiopia (≥40% and 100%, respectively). Reductions in efficacy of standard LLINs is of particular concern considering all nets tested were new, unused and adhered to target dose ranges. Such data from multiple mosquito populations and tools provides a good basis for informing control decisions in the absence of more comprehensive comparative data.

Keywords:*LLIN, efficacy, Anopheles, cone bioassays*

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O602TH06

Medical Entomology

O602

Implications of larval density-dependence processes on malaria vector control strategies in Kenya

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Back ground: Malaria vector management is an integral part of malaria control strategies in Sub-Saharan Africa. For any successful planning and implementation of vector control strategy prior understanding of the vector bionomics is crucial in order to design and optimize control measures based on when, where and how malaria vectors should managed. Objective: In the current study, density?dependent processes during larval stages in Anopheles gambiae s.s larval habitats and their contribution to population regulation were evaluated. Methodology: A series of experiments were done in which An. gambiae s.s densities and cohort structure were manipulated in the field in Kilifi, Coastal Kenya using artificial habitats set in semi-field conditions. Results: Simple general linear models analysis showed considerable variation in mosquito survival rates, measured by the probability of successful pupation across different densities with 23-31% of the differences in the survival being attributed to mosquito density. The form of the density-dependence was nearly linear when the log-odds of survival were plotted against log density and a two-fold increase in density resulted in a reduction of the log-odds of survival. Higher larval density not only increases mortality but lengthens the time the mosquito requires to reach the pupal stage by 3-4 days. Mosquitoes emerging from high density breeding sites tend to be smaller and consequently have reduced survival rate and fitness. Conclusion: In conclusion strong density-dependence pervades in mosquito larval populations through larval competition with implications on the approaches employed in the control of malaria vectors in natural settings.

Keywords:*Anopheles gambiae, Larvae, Density-dependent, Models*

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O602

Evaluating the potential of the sterile insect technique for malaria control: Relative fitness and mating compatibility between laboratory colonized and a wild population of *Anopheles arabiensis*

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Background: Successful suppression of a target insect population using the sterile insect technique (SIT) partly depends on the premise that laboratory insects used for mass rearing are genetically compatible with the target population, that mating competitiveness of laboratory reared males is at least comparable to that of their wild counterparts, and that mass rearing and sterilization processes do not in themselves compromise male fitness to a degree that precludes them from successfully competing for mates in the wild. This study investigated the fitness and sexual cross-compatibility between samples of field collected and laboratory reared An. arabiensis under laboratory conditions. Results: The physiological and reproductive fitness of the MALPAN laboratory strain is not substantially modified with respect to the field population at Malahlapanga. Further, a high degree of mating compatibility between MALPAN and the Malahlapanga population was established based on cross-mating experiments. Lastly, the morphological characteristics of hybrid ovarian polytene chromosomes further support the contention that the MALPAN laboratory colony and the An. arabiensis population at Malahlapanga are genetically homogenous and therefore compatible. Conclusions: It is concluded that presence of a perennial and isolated population of An. arabiensis at Malahlapanga presents a unique opportunity for assessing the feasibility of SIT as a malaria vector control option. The MALPAN laboratory colony retained sufficient enough measures of reproductive and physiological fitness to present as a suitable candidate for male sterilization, mass rearing and subsequent mass release of sterile males at Malahlapanga in order to further assess feasibility of SIT in a field setting.

Keywords:*Sterile Insect Technique, Anopheles arabiensis, malaria vector control*

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O602TH08

Medical Entomology

O602

Cow dung, medicinal plants and intermittent irrigation strategy for anopheles mosquito management in Ahero rice paddies

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Human health is a major concern in many parts of the world. Although irrigated farming has been used to increase food production, this has aggravated the problem of mosquito vectors and subsequent increases in malaria transmission. Integrated vector management targeting both the adult and immature stages has been adopted with renewed interest in larval management with the aim of making it more effective. Targeting mosquito larval habitats like irrigated rice agro-ecosystems of Ahero calls for research to evaluate affordable, environmentally friendly approaches such as cattle dung, intermittent irrigation and neem (Azardirachta indica). This research investigated the effect of cow dung, neem, and intermittent irrigation on immature stages of anopheles mosquitoes. Laboratory and field experiments were carried out in Ahero under different irrigation regimes on malaria vector. Field experiments were designed using a mixed model approach with three treatments replicated four times per block. The highest mosquito larval densities were recorded in Ammonium sulphate treated sub-plots. Results for laboratory experiments indicated that cow dung and neem individually caused significant mortality of immature stages of mosquitoes. A combination of cow dung and neem caused higher mortality than cow dung, neem alone but the difference was not significant. A reduction in the number of larvae in experimental plots with cow dung and neem was recorded suggesting that larvae and adult mosquitoes may be reduced by cow dung and neem. Intermittent irrigation, where draining of the rice paddies was associated with the lowest mosquito larvae could be adopted in mosquito management.

Keywords:*mosquito, rice, cow dung, neem, intermittent irrigation*

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O602TH09

Medical Entomology

O602

Performace of new products against *Aedes aegypti/albopictus* under laboratory and field conditions: A preparatory approach following the 2011 dengue epedemic in Pakistan

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Pakistan in particular Lahore faced one of the biggest epidemic of dengue ever reported in the world. Vector breeding and the future threats of the disease outbreak alongwith studies on latest products with more environmental friendly impacts were explored. The trials have revealed effectiveness of the products to kill the mosquito populations with minimal impact on the environment. A total of five products containing three Bti formulations one IGR and a physical barrier were tested and a comparison in terms of their performance against all the life stages were reocrded. The results with Aquatain AMF a silicone based polymer (Polydimethylesiloxane PDMS) reveal that the product when used against Aedes aegypti provided 100% mortality of the pupal and 4th instar larvae and 85% mortailty of 3rd after 12 hours of application. 30% mortality was recorded in 1st and 2nd instars, however 90% mortality was recorded in these larvae when they grew up to 3rd and 4th instars. Percent mortality with respect to time in Culex populations were different from the Aedes. Similarly mortality from TMOF-Bti, Mosquito Dunks, Sumilarv (IGR) and MOSTAB-Bti have revealed higher percentages. The performance of Bti products was effected at lower temperatures, but genarlly all of the products performed well at temperatures above 20ºC.

Keywords:*Aquatain, Tmopf-Bti, Dunks, Sumilarv and Aedes mosquitoes*

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O602TH10

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O602

Role of public participation and interventions in dengue management

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Abstract: Dengue and the dengue hemorrhagic fever are caused by two known species of mosquitoes Aedes aegypti and Aedes albopictus that have extended their ranges from Asian region to other continents through trade and transportation. Both the species have the capacity to vector all the four serotypes (DEN1, DEN2, DEN3 and DEN4) and ultimately transfer the virus to the people after extrinsic incubation period of 4-6 days. At present Pakistan in particular Punjab stands as the target site for dengue. Climatic changes, unplanned urbanization and the growing environmental concerns may further lead to the expansion of vector's ranges and finally the prevalence of the disease. Places which have shown sever epidemics have also opened doors to other vector borne issues and the continuous application of insecticides may select the resistant forms already reported from Multan, Faisalabad and Lahore to other parts of the Punjab and the country. It is an established fact that not only the interventions and research can prevent the disease outbreak but it is the public participation activated through communication and change in behavior (COMBI) which can make the difference. This paper therefore, highlights the areas of our future strategies for combating with any of the vector borne issues. It emphasizes on the concept that there are multiple options available for the management of vector and if all the components are followed an outbreak can ultimately be prevented.

Keywords:*Aedes, vector, IVM, Epidemic management strategy, Dengue*

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O602TH11

Medical Entomology

O602

Repellency of cassia bark, Eucalyptus and Star anise oils and their major constituents to *Leptotomobidium pallidum* (Acari: Trombiculidae)

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Repellents can be effective tools for protecting individuals from mite bites and mite-borne diseases because the mite larvae live in micro spots called mite-islands and control of the chigger mites with residual spray is very difficult. The most widely used repellent products are currently based on N,N-diethyl-3-methylbenzamide (Deet). It is estimated that approximately one-third of the people in the United States use Deet-containing products in various formulations. However, occasional toxic effects of Deet have been reported that include central nervous system depression, urticaria, contact dermatitis, or potential encephalopathic toxicity. There is, therefore, a critical need for the development of more effective new repellents and repellent formulations. An assessment is made of the repellency of cassia bark, eucalyptus and star anise oils and their major constituents against Leptotrombidium pallidum larvae. Results were compared with those of conventional repellents DEET, IR3535 and permethrin. Based on RC50 values, cassia bark oil was the most effective repellent and was 2.5, 12.6 and 15.0 times more effective than Deet, IR3535 and permethrin respectively. Star anise oil was more pronounced in the repellency than eucalyptus oil. The repellency of star anise oil, eucalyptus oil and Deet did not differ significantly from each other. Star anise and eucalyptus oils were significantly more effective than either IR3535 or permethrin. In the light of global efforts to reduce the level of highly toxic synthetic repellents, the three essential oils and their major constituents described herein merit further study as potential repellents for the control of house dust mite populations.

Keywords:*Culex tritaeniorhynchus, enzyme microplate assay, insecticide resistance, detoxifying enzyme*

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O602TH12

Medical Entomology

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Histopathological effects of pellitorine on larval midgut epithelium and anal gills toward *Aedes aegypti* (Diptera:Culicidae)

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The histopathological effects of pellitorine on larval segments of third instars of Aedes aegypti were examined by transmission electron microscopy. Results were compared with those of deltamethrin. At a concentration of 5 mg/L, pellitorine was targeted mainly on midgut epithelium and anal gills, indicating variably dramatic degenerative response of the midgut through a sequential epithelial disorganization. The anterior midgut was almost entirely necrosed, bearing only residues inside the plasma membranes on the basal lamina. The compound has shown complete damage of all glandular cells of anal gills, because cuticle damage was occurred by pellitorine. The histopathological effects 2.5 mg/L of deltamethrin on the different regions of the midgut clearly indicate the epithelial disorganization and delocalization of cell organelles which have been correlated with their morphofunctional status. Based on these results, pellitorine merit further study as a potential larvicide with a specific target site and mode of delivery or a lead molecule for the control mosquito populations.

Keywords:*Aedes aegypti, Pellitorine, Deltamethrin, Histopathology, Midgut, Anal gills*

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O602TH13

Medical Entomology

O602

Insecticide resistance in dengue and malaria vectors and consequences for vector control

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The use of insecticides in public health has increased dramatically in the past decade through the scale up of insecticide treated net distribution programmes, indoor residual spraying and larviciding campaigns or space spray operations. Inevitably, the major malaria and dengue vectors have developed resistance to these insecticides and the resistance alleles are now spreading at an exceptionally rapid rate worldwide. Although decades of extensive research and substantial progress have been made on understanding the causes of insecticide resistance, remarkably few studies have focused on the impact of resistance on current vector control activities. Here, we will address the current distribution of insecticide resistance in dengue and malaria vectors in the tropics and discuss its implications for vector control in different settings (South East Asia, Africa and Caribbean). The evidence that insecticide resistance represents a major threat for the control of dengue and malaria calls for new vector control strategies in the coming years

Keywords:*malaria, dengue,vector control, insecticide, resistance*

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O602TH14

Medical Entomology

O602

Sumilarv®0.5G, Promising insecticide for the control of *Anopheles gambiae*

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Malaria vector control with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has resulted in a significant decline of malaria in Africa. However, recent reports have shown both behavioural avoidance and physiological resistance to insecticides by vectors. Thus, the need to explore and integrate other tools for malaria vector control. Larval source management is a proven tool for control. Difficulties with larviciding are (1) interventions are based at targeting all aquatic habitats and (2) current larvicides have short residual activity requiring weekly application which necessitate large labour and large amounts of larvicides is not cost effective especially in Africa. Thus the need to evaluate persistent larvicides and study sound application strategies. Sumilarv is a persistent larvicides that with great potential for mosquito control. Bioassays showed that Sumilarv was ten times more effective that microbials for Anopheles control. Sumilarv application at 0.05 ppm a.i resulted in 80% emergence inhibition for upto 6 weeks in standardized field conditions. Evaluations of the optimum dose of Sumilarv identified in the standardized field tests are on-going in an area of focal malaria transmission in Western Kenya. Preliminary results from the first three months of field testing indicate the three week application results on average in 71% emergence inhibition of malaria vectors from treated sites. The use of residual larvicides has a risk of vector production from untreated habitats created or filled with water after larvicide application. Intensive monitoring and sampling of sites is ongoing to estimate this risk in the dry and rainy seasons.

Keywords:*Residual*

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O602TH15

Medical Entomology

O602

Scientists and public involvement: A consultation on the relation between malaria, vector control and transgenic mosquitoes

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Among the hopes for vector-based malaria control, the use of transgenic mosquitoes able to kill malaria parasites is seen as a potential way to interrupt malaria transmission. While this potential solution is gaining some support, the ethical and social aspects related to this high-tech method remain largely unexplored and underestimated. Related to those latter points, the aim of the present survey is to determine how scientists working on malaria and its vector mosquitoes perceive public opinion and how they evaluate public consultations on their research. This study has been performed through a questionnaire addressing questions related to the type of research, the location, the nationality and the perception of the public involvement by scientists. The results suggest that even if malaria researchers agree to interact with a non-scientific audience, they (especially the ones from the global North) remain quite reluctant to have their research project submitted in a jargon-free version to the evaluation and the prior-agreement by a group of non-specialists. The study, by interrogating the links between the scientific community and the public from the perspective of the scientists, reveals the importance of fostering structures and processes that could lead to a better involvement of a non specialist public in the actual debates linking scientific, technological and public health issues in Africa.

Keywords:*Malaria, Mosquito, Transgenic, Public health, Social science, Public engagement*

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O602TH16

Medical Entomology

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Technical basis for deployment of a combination long lasting insecticidal net in areas of pyrethroid resistance

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Increasing reports of pyrethroid resistance in many malaria-endemic countries have highlighted the need for tools with increased efficacy. A review of the evidence to support the function and efficacy of the first combination long lasting insecticidal net (LLIN) will be given. The rationale for the design of the tool will be presented including data to show that the net meets current WHO criteria for an LLIN. Results from experimental hut studies in 4 African countries will be presented showing increased efficacy against pyrethroid resistant malaria vectors. Differences in efficacy from country to country will be discussed in the context of the background resistance present in the target population where these studies were conducted. A comparison of the efficacy of the combination LLIN with a mono-treated LLIN at the village level will be presented. Finally, the utility of modelling to inform on community level effects expected with new products will be discussed. The type and scope of evidence required for decision-makers when new tools are introduced will be considered, both at the country level and at a global level.

Keywords:*pyrethroid resistance, LLIN, experimental hut, village study, modelling, review*

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O603F01

Medical Entomology O603

Bacteria and fungi species associated with digestive system of *Blattella germanica* L. collected in spring and autumn from various parts of a public hospital in Mashhad, Iran.

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With regards to the role of cockroaches in transporting and distributing of various disease agents in remedial and hospital environments, the present study aimed to study the bacteria and fungi flora associated with the digestive system of Blattella germanica L. collected in spring and autumn (2010-2011) from different sections of a general hospital in Mashhad. Ten cockroaches were captured from each of 5 locations. After dissection of digestive system under sterile conditions, the extracts were incubated in EMB and Blood Agar medium. Bacteria and fungi were identified by clinic of microbiology of Imam Reza hospital of Mashhad. In autumn, the most prevalent bacterium was Enterococcus spp. (78%). Other bacteria consisted of Coagulase Negative Staphylococci, Staphylococcus aureus, Streptococcus spp., Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa and Gram positive bacilli. In this season, 8 (out of 50) cockroaches were contaminated with fungi (16%), namely Penicillium spp. (50%), Mucor spp. (37.5%) and Aspergillus spp. (12.5%). In spring, the most prevalent species was Enterococcus spp. (48%). Other bacteria in this season consisted of Coagulase Negative Staphylococci, S. aureus, E. coli, Klebsiella spp., Tettragen spp., Citrobacter frondi, Serratia marsesens and Gram positive bacilli. In this season, 6 cockroaches were contaminated with fungi (14%), namely Penicillium spp. (14%), Mucor spp. (57%), Aspergillus spp. (14%) and Candida albicanse (14%). The results suggested that for reducing the risk of transmission of various disease agents, suitable control measures should be applied to manage the population of cockroaches in hospitals.

Keywords:*Spring, Autumn, Bacteria, Fungi, German cockroach, Hospital, Mashhad*

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O603F02

Medical Entomology O603

Expression profile analysis of detoxification genes in different life stages of *Anopheles funestus*

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Anopheles funestus is a major vector of malaria in the southern African region and resistance to pyrethroids has been recorded in populations of this species. Quantifying differential expression of these genes at the different life stages of a mosquito will provide some insight into their role and function. Variation in the expression of these enzymes through the developmental stages in An. funestus is currently undocumented. The aim of this study was to therefore determine the transcription profile of the detoxification genes in the first instar, fourth instar and 3-day old adult mosquitoes, using the An. gambiae detox chip. Microarray analysis including dye labeling, hybridization, scanning and data analysis were performed. Results showed that in the fourth instar larvae and 3-day old adult comparison, 35 genes were overexpressed in the fourth instar larvae and seven genes in the adult stage. The comparison between first and fourth instar larvae, 67 genes showed increased gene transcription in the first instar larvae and nine genes in the fourth instar larvae. A total of 91 genes were overexpressed in the first instar larvae and five in the adults, when these two life stages were compared to each other. The differentially expressed genes in all life stages consisted mostly of cytochrome P450s, esterases, glutathione S-transferases and other additional genes. This study provided vital information regarding genes potentially involved in pyrethroid resistant and is the first to provide metabolic or detoxifying transcription gene information in An. funestus.

Keywords:*Anopheles funestus, first instar, fourth instar, cytochrome P450s, esterases, glutathione-S-transfer*

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O603F03

Medical Entomology

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Physiological tolerances and species distribution modelling of two malaria vectors

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A major concern associated with climate change is how changing rainfall patterns and temperature regimes will impact on the possible spread and distribution of vector-borne diseases (VBDs). In southern Africa, malaria is a VBD responsible for almost one million deaths annually. Information on the physiology of the mosquito vectors of the disease is largely lacking, despite its importance in determining the niche occupied by a species. Thermal tolerance, desiccation and starvation resistance, as well as development rate-temperature relationships were investigated for two of the most prominent malaria vectors in Africa: Anopheles arabiensis and An. funestus. On the whole, data suggest that females (the sex responsible for disease transmission) are more tolerant of environmental stress and changes to environmental conditions than males. Additionally, the ability of the immature forms (larvae and pupae) to withstand significantly greater temperatures than adults, suggests that breeding populations of Anopheles mosquitoes might be positively impacted by overall warming of global temperatures. Preliminary species distribution models indicate that at the boundaries of the malaria vectors' current distribution, and in regions where altitude and, by proxy, minimum temperatures, have been limiting to distribution, the potential for resurgence or emergence of malaria exists with changing climates.

Keywords:*Malaria, climate change, physiology, distribution modelling*

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O603F04

Medical Entomology O603

Susceptibility of *Anopheles barbirostris* complex to *Plasmodium falciparum* and *Plasmodium vivax* in Thailand

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Most Anopheles mosquitoes belong to species complexes is differences in malarial vector-competence among sibling species members. Recently, based on morphological, cytogenetic and crossing experiments and molecular investigation, at least 5 sibling species have been discovered within the taxon An. barbirostris in Thailand, i.e., An. campestris-like and An. barbirostris species A1, A2, A3, and A4. This study investigates the susceptibility of 5 sibling species to malaria parasites in laboratory conditions. Nine colonies of five sibling species members of An. barbirostris complexes were infected experimentally with P. falciparum and P. vivax. They were then dissected 8 and 14 days after feeding for oocyst and sporozoite rates, respectively, and compared with An. cracens. The results revealed that all of the mosquitoes in this experiment were completely refractory to P. falciparum. Likewise, An. campestris-like Forms E (Sa Kaeo) and F (Ayuttaya), and An. barbirostris species A4, were non-potential vectors for P. vivax. The lower than normal development of oocysts and sporozoites recovered from An. barbirostris species A1, A2 and A3, demonstrated that these species were low potential vectors for P. vivax. Only An. campestris-like Forms B and E (Chiang Mai) were high-potential vectors for P. vivax because normally high development of oocyst and sporozoite rates were obtained.

Keywords:*Anopheles barbirostris complexes, malaria susceptibility, Plasmodium falciparum, Plasmodium vivax*

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Medical Entomology

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Possible factors that induce nocturnal activities of blow flies (Diptera: Calliphoridae)

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The ages of blow fly larvae that appeared on the corpses were used to estimate the post-mortem interval (PMI) of the victims in the criminal investigations. Thus the time gap between the blow fly oviposition and the death of a victim is a critical issue. Although blow flies have long been thought that they could not lay eggs at night, there are still several illustrations of nocturnal oviposition have been reported. Due to the limitation of oviposition sites for necrophagous blow flies, the locomotor activities such as searching and locating ability of the blow flies could highly affect their oviposition. According to the previous studies, circadian rhythm can regulate the fly locomotor activity and eye sensitivity. Since the blow flies are not active at night, our study is trying to find out if there is any possible factor that could break down the circadian rhythm of blow flies and thus induce their nocturnal activities or even the nocturnal oviposition under laboratory conditions. Our results suggested that the light is a possible factor that significantly increased the activity of blow fly at nighttime, but the temperature increase is otherwise not. If we simultaneously treated the blow flies with light and higher temperature at nighttime, there was no additional effect to their activities. These results may help to understand the nocturnal behavior of blow flies as well as to adjust the nighttime PMI estimation.

Keywords:*Calliphoridae, locomotion, nocturnal oviposition, forensic entomology*

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O603F06

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O603

Gregarious oviposition of blow flies (Diptera: Calliphoridae)

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The ages of necrophagous blow fly larvae are commonly used to estimate the post-mortem interval (PMI) of victims or animals in forensic entomology, thus the blow fly oviposition, the starting point of a fly life history, becomes an important factor in those applications. Oviposition behavior also plays a very important role in the evolutionary adaptation for a species. Under the limited carrying capacity of the larval resource, the clutch size produced by parental females may influence the progeny competition and fitness. Therefore, insects seldom show gregarious oviposition behavior, which means several females oviposite at the same site or even in a cluster. But the blow flies seem to be one of the exceptions; in the field, several blow flies usually lay large amount of eggs on the same carcass. To understand the gregarious effects on blow fly oviposition, we test the fecundity of Chrysomya megacephala (Fabricius) (Diptera: Calliphoridae) under different coexisting amounts and types of blow fly companies. Results showed the blow flies rarely lay egg when alone. And gregariousness not only induced the oviposition behavior but also increased the clutch size for each individual. In addition, different types of blow fly companies, such as different sexes or species, could also affect the oviposition performance. Our study herein proposes the significant effects of gregarious behavior on oviposition, and we presume these results may have potential usage in forensic investigation, especially when using blow fly thermo history on PMI estimation.

Keywords:*Chrysomya megacephala, gregarious oviposition, blow flies, forensic entomology*

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Body size measurements as quality indicators in *Aedes aegypti* mosquitoes destined for field release

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There is increasing interest in rearing modified mosquitoes for mass release to control vector-borne diseases, including release of Wolbachia-infected Aedes aegypti mosquitoes for suppression of dengue. Successful manipulations of natural populations require the release of high quality mosquitoes. Potential simple quality indicators are body size and related measurements. Here we test if size and wing/thorax ratio, (thought to be related to flight ability), can be related to field fitness of Wolbachia-infected A. aegypti. Released mosquitoes were much larger on average than field-collected populations and the variance was much smaller. Wing/thorax ratios had lower overall variation with less distinct differences between released and field mosquitoes. We monitored the 2011 trial release of Wolbachia-infected A. aegypti and found that over time, Wolbachia-infected A. aegypti bred in the field achieved similar variance to the wild population. Based on the size differences of field and release mosquitoes, we estimated that the survival of released mosquitoes was comparable to field mosquitoes. By collecting infected adult females from sticky traps during an open field release, we showed that within the limited variance of field cage reared mosquitoes, there was no evidence for size-related selection, either in terms of females feeding and reaching oviposition sites or survival. However within uninfected field mosquitoes collected from traps, gravid females tended to be larger than others suggesting an advantage for larger females. These findings suggest that, for females, size is a useful fitness indicator and rearing conditions should aim for large size without unduly affecting wing size/thorax ratios.

Keywords:*Aedes aegypti, Body size, Quality control, Fitness, Field release*

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O603F08

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O603

Effectiveness of Bti-based product from Bti cells cultivated by using glutamic acid mother liquor, a waste of monosodium glutamate production

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The present study was aimed at utilization of glutamic acid mother liquor (GM1), a waste of monosodium glutamate production, as cultivation medium for B. thuringiensis subsp. israelensis (Phrae strain) and assessment on the larvicidal activity of Bti-based product against Aedes aegypti larvae. Results indicated that initial pH of 7.2-7.8, temperature of 30-34 oC and aeration of 150-250 rpm were appropriate environment for cultivation of candidate strain. Among a dilution series of GM1, GM1 at 1:8 exhibited the highest amount of cell mass of 3.976 g/l while those of GM1 at 1:64 gave the lowest of 0.635 g/l. The highest larvicidal activity against Aedes aegypti was found from GM1 at 1:64 while those of GM1 at 1:8 showed the lowest. Increase of cell mass by supplementing 0.2 g/l of glucose in GM1 at 1:64 was promised. A submerge fermentation under weather temperature and 282 rpm indicated that steady state was recorded at 39th hours after inoculation. pH varied from 6.59-8.18. The cells during 18-30 hours of age showed the best larvicidal activity with non statistical difference. The potency of the cells from GM1 plus glucose was 14,370 ITU/mg. A tablet form of product coded NIH-BT 2011 was performed for simulated field evaluation. After 7 months of monitoring the shelf life of product, reduction of larvicidal activity was not yet noticed. The duration that product provided more than 90% larval mortality when water in the containers was 25, 50 and 90% removed and replaced, were 45, 40 and 25 days, respectively.

Keywords:*Bti product, cultivation medium, Aedes aegypti*

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O603F09

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The effect of blood feeding on gene expression in insecticide resistant *Anopheles funestus*

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Indoor spraying of houses with residual insecticides (IRS) is the mainstay of many malaria vector control programs in Africa. IRS targets resting adult female Anopheles mosquitoes before or after blood feeding. During blood feeding, the mosquito may ingest toxic xenobiotics present in the host's blood. In the resting period after feeding, many processes are initiated to assist in the digestion and assimilation of the blood nutrients. Since the regulation of xenobiotic (including insecticides) detoxification enzyme systems is likely to be altered in response to the ingestion of blood, this study aimed to investigate the effect of a blood meal on insecticide tolerance in insecticide resistant and susceptible southern African strains of the major malaria vector Anopheles funestus. We have demonstrated that blood fed An. funestus, carrying a resistance phenotype, are able to tolerate higher doses of insecticide than their unfed counterparts. Microarray studies on these blood-fed mosquitoes showed a general increase in delta class glutathione-s-transferase (GST) expression. An An. funestus orthologue of the GSTD3 gene was investigated as a target for qRT-PCR, but the results were inconclusive. Since the pyrethroid resistant phenotype of southern African An. funestus has been linked to the over-expression of the duplicate copy gene CYP6P9, the expression of CYP6P9 and CYP6P13 were quantified. Both genes showed a small, but significant, increase in response to the blood meal. The increased expression of these major effect CYP6 genes in response to blood feeding may be responsible for the increase in insecticide tolerance seen in the bioassays.

Keywords:*Anopheles funestus, blood meal, insecticide resistance*

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O603F10

Medical Entomology

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Characterization of akirin in the South African malaria vector *Anopheles arabiensis*, and its importance as a novel tool to serve as a vector control intervention.

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The malaria parasite (Plasmodium spp.) is transmitted to humans by various Anopheles spp., with over 90% of human infections occurring in sub-Saharan Africa. The Anopheles gambiae complex compromises of seven species, whereof two species are considered as major malaria vectors in tropical Africa, An. gambiae s.s. and An. arabiensis. In order to enhance South Africa's malaria control capacity, additional vector control tools are investigated for their practical incorporation into existing control programmes. Other researchers have shown that a tick specific vaccine, subolesin, provides protection against tick-borne pathogens. This study aimed to characterize the orthologous gene, akirin, in An arabiensis, and phylogenetically compare it to other closely related species, i.e. An merus, An. gambiae and An. quadriannulatus. Preliminary results achieved have confirmed a high level of sequence identity between akirin in An. arabiensis and other insects. The potential of akirin, as a tool for further use in immunization studies on the fitness of An. arabiensis will be examined. It is expected to reduce mosquito survival, feeding rate and reproduction after feeding experiments on a host vaccinated with recombinant tick and Aedes albopictus akirin. The application for in vivo gene silencing and phenotype assessment of An. arabiensis feeding on subolesin using double-stranded RNA (dsRNA) is discussed.

Keywords:*Anopheles arabiensis, akirin, sequences, vaccination*

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O603F11

Medical Entomology O603

Differential proteomic study of *Plasmodium falciparum*-infected and non-infected salivary glands of *Anopheles gambiae*, to a biomarker of infective bites

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Malaria is caused by parasites Plasmodium genus transmitted by Anopheles mosquitoes during the blood feeding. During this blood meal, saliva and parasites are injected into the vertebrate host skin. Salivary molecules possess a pharmacologic role allowing correct blood feeding and counteracting the acquired host immune response throught their immunomodulation properties. It has been demonstrated that the expression of salivary proteins of Anopheles gambiae infected by Plasmodium berghei are modified. Here we investigate what modifications in salivary glands of An. gambiae occur during the infection by wild P. falciparum. To assess this question we compare salivary extracts of An. gambiae infected and uninfected by proteomic approach. Experimental infections of An. gambiae by wild P. falciparum are carried out in Cameroon and salivary glands are dissected 14 days post-infection. The quantitative-PCR allowed to quantify the P. falciparum infection into the salivary glands. Two-Dimensional Differential Gel Electrophoresis (2D-DIGE) are underway with different pools of non-infected versus infected salivary glands. Preliminary results showed that several components are over- or down-expressed during the infection. Mass spectrometry analysis will reveal differentially expressed proteins. These results suggest that the parasite surely induces modifications of salivary proteins expression, certainly favoring its transmission. This study will give us more knowledge about the relationships between the vector and its host and may lead to the developpment of new tools to better evaluate the risk of malaria transmission, like an immuno-epidemiologic biomarker. These observations represent important new applications for evaluation of vector control strategies

Keywords:*Anopheles gambiae, Plasmodium falciparum, salivary proteins, biomarker*

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O603F12

Medical Entomology O603

Electric nets and sticky materials for studying oviposition behaviour of gravid malaria vectors

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Indoor sampling of Anophels gambiae s.l., the major malaria vector in sub-Saharan Africa, has become difficult due to continued insecticide use inside houses. Alternative sampling tool need to be developed to collect vectors outdoors. One target could be a gravid female in search for an oviposition site but little is known how this vector approaches and selects suitable habitats and how eggs are deposited. In order to study this behaviour we developed electrocuting nets (e-nets) and various sticky materials under semi-field condition using insectary ? reared An. gambiae s.s.. E-nets proofed to work well for the collection of anophelines orienting towards an odour source when the nets were electrified in such a way that no visual sparks were generated ($p<0.001$).Furthermore, it was necessary to develop a collection device below the e-nets where stun mosquitoes were contained which was by itself not attracting mosquitoes. Three different sticky materials were tested, a transparent film, a yellow film and a black netting with insect glue. The transparent and black surfaces were highly attractive for gravid anophelines as compared to the yellow film ($p<0.001$) and could play a potential role as gravid traps in the future. Sticky materials and detergents were used to study landing on the water surface or on the edge of habitats for oviposition. Significantly, more An. gambiae females land directly on the water surface to deposit their eggs than on the edge of habitats ($p<0.001$). Implications of the results for the development of gravid traps will be discussed.

Keywords:*Electric nets, sticky materials, Anopheles gambiae, oviposition*

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Medical Entomology

O603

Development of *Plasmodium berghei* in *Anopheles funestus*

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Anopheles funestus is a major malaria vector in Sub-Saharan Africa, efficient in its vectorial capacity and is highly anthropophilic and endophilic. Despite its importance, research on the species has been severely limited due to a lack of laboratory colonies until 2000. An infection model using Plasmodium berghei with An. funestus was successfully established recently, but details of the parasite development remains largely unknown. To investigate the development, adult females from the base An. funestus colony FUMOZ was allowed to feed on C57/Black mice infected with ANKA strain of P. berghei. The bloodfed females were maintained at 21±1°C, 85±10% relative humidity and provided with 10% (w/v) sucrose water for sustenance. Midgut and salivary glands dissection were performed to determine the appearance of oocysts and sporozoites in the two organs respectively, and to quantify the infection. Oocysts were seen 10-12 days after the infected bloodmeal, and sporozoite appeared 18-21 days post bloodmeal. Average oocyst number was around 25, leading to 2-3 000 sporozoites in the salivary glands. Infected females were able to initiate cyclical transmission upon feeding on a healthy mouse, demonstrating the viability of the parasite. The results showed that P. berghei development in An. funestus is longer than in other vectors. Quantification of the life stages indicate that Anopheles funestus is not as an efficient vector of P. berghei compared to An. Stephensi, but is better than An. quadrimaculatus. Nonetheless, it is still a viable vector, and the parasite development data will be of use for future studies.

Keywords:*Anopheles funestus, Plasmodium berghei, infection, parasite development, oocysts, sporozoites*

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Medical Entomology

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Aedes aegypti and *Aedes albopictus* breeding recepticles and their association with the number of dengue cases reported

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Aedes populations were collected during the epidemic period starting from July 2011 and going up to the end of Nov 2011. Both indoor and out door survveillance was done and all indices were recorded. The vector was breeding extensively in all major recepticles. The most potential out door breeding site included the automobile tyres, construction sites, plastic wrappers, scrap dumping areas. While the major indoor breeding sites included those houses where the water supply was limited to few hours and water containers were used for storing water either for consumption or washing. The water containers along with the underground and over head water tanks were found positive for the Aedes larvae. As for the percentage of the species dominance is concerned the Aedes albopictus was more confined to out door while Aedes aegypti was dominating the indoor premises like the refrigerator trays, room coolers, roof tops blocked spill ways on roof tops etc . The population peaks were recorded from end July 2011 and continued till mid October 2011, there after the population declined to very few breeding sites. No population was collected during the months of December 2011 to end of February 2012 despite the early spring showers in end of February 2012. First population was reported within the houses in water storage containers in the first week of March 2012 that included the Aedes aegypti larvae. A strong association of the breeding sites and the number of dengue infected cases was recorded during the 2011 epidemic.

Keywords:*Aedes albopictus, Ae. aegypti, breeding sites, positive containers*

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Population genetic structure of the major malaria vector *Anopheles funestus* s.s. and allied species in southern Africa

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Anopheles funestus sensu stricto is one of the major malaria vectors in sub-Saharan Africa belonging to a group of ten morphologically similar species. The population structure of An. funestus s.s. based on mitochondrial DNA data led to the description of two cryptic subdivisions, clade I widespread throughout Africa and clade II known only from Mozambique and Madagascar. We investigated five common members of the Anopheles funestus group including An. funestus s.s. clades I and II in order to determine relationships within and between species. The population genetic structures of An. funestus s.s., An. funestus-like, An. parensis, An. rivulorum and An. vaneedeni from South Africa, Mozambique and Malawi were assessed using NADH Dehydrogenase subunit 5 (ND5) and Cytochrome Oxydase I (COI) within and between populations. The phylogenetic trees for both genes revealed three main lineages: 1) An. rivulorum, 2) An. funestus-like and 3) Anopheles funestus subgroup including An. funestus s.s. clades I and II, An. parensis and An. vaneedeni. Interestingly, An. funestus-like, An. parensis and An. vaneedeni exhibited two clades each. This is the first report comparing An. funestus group species including An. funestus clade I and II and the new species An. funestus-like. Anopheles funestus clade I is separated from the rest members of the An. funestus subgroup within the lineage and An. funestus-like is distinctly distributed from the other species in this study. Further investigations are needed to determine what these results mean in terms of their specific status.

Keywords:*Anopheles funestus, ND5, COI, Phylogeny*

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Medical Entomology

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DNA barcoding of the mosquito genus *Culex* (Diptera, Culicidae)

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Culex is a large cosmopolitan genus of mosquitoes. Several species are vectors for arboviruses (e.g. West Nile and Japanese encephalitis viruses) and filarial worms that cause disease in humans. To facilitate an effective management of both mosquitoes and diseases, accurate identification of vectors is a key element. Within the framework of the Mosquito Barcoding Initiative, which aims to produce identification tags for 80% of all currently recognized species of Culicidae (3531 species), and in conjunction with an ongoing integrated morphological and molecular systematics study of the tribe Culicini at The Natural History Museum in London, we carried out barcoding of Culex species to provide reliable identification tools. To achieve our sampling, we relied heavily on archive specimens in major museums of the world. We investigated the utility of the COI barcode region for species identification across the genus Culex and other members of the tribe Culicini. We discuss the power of resolution of DNA barcoding and its limitations for reliable identification of specific and subspecific taxa and members of cryptic species complexes, as well as the use of barcodes as a possible tool to uncover genetic variability that may lead to the discovery of new taxa.

Keywords:*barcoding, Culicidae, Culex*

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O603F17

Medical Entomology O603

Effect of Ketamine hydrochloride on the development of a forensically important blow fly *Chrysomya megacephala* (Fab.) (Diptera: Calliphoridae)

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Entomotoxicology is a specialized area of forensic entomology that deals with the qualitative and/or quantitative determination of toxic substances (e.g. drugs, environmental pollutants, pesticides) in insects and other arthropods feeding on human remains. These natural scavengers can serve as a source for toxicological analysis when the tissues or body fluids normally used for this purpose are not available. Qualitative determination of toxic substances and drugs can add information on both the cause of death and the geographical area where the cadaver had been lying. Since toxic substances can modify the biological cycle of arthropods, their quantitative determination provides an additional parameter for evaluating the post-mortem interval (PMI). Ketamine hydrochloride is a Schedule 'H' drug used in human and veterinary medicine. It produces dissociative anaesthesia and is commonly abused as a recreational drug. It has also gained notoriety as a 'date-rape' drug and may be found in cases of drug overdose related deaths or in suspected drug assisted rape and murder assaults. The objective of this study was to analyze its effect on the development of immature stages of Chrysomya megacephala (Fabricius) reared on rat tissues containing Ketamine hydrochloride administered antemortem. The presence of Ketamine hydrochloride could be detected through Gas chromatography (GC) in all the rat samples and in almost all fly samples in the experiment. Results show good correlation between the quantity of Ketamine hydrochloride administered and the rate of development of the immature stages of this forensically important fly.

Keywords:*Forensic entomology, Ketamine hydrochloride*

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O603F18

Medical Entomology O603

Development of an immuno-epidemiological biomarker for evaluating the exposure to the bites of *Aedes aegypti* mosquito.

Emmanuel Elanga Ndille[1], Doucoure Souleymane[2], Francois Mouchet[3], Damien Georgia[4], Pape Drame[5], Herbert Noukpo[6], Nicolas Moiroux[7], Dorothee Misse[8], Martin Akogbeto[9], Vincent Corbel[10], Marie-claire Henry[11], Fabrice Chandre[12], Thierry Baldet[13], Franck Remoue[14]

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Background: Measuring human exposure to arthropod bites, would allow estimating transmission risk of certain vector-borne diseases. Aedes aegypti mosquito is the primary vector of arboviruses such as dengue, Chikungunya and yellow fever. The present study aim at evaluating human specific antibody (Ab) IgG response to Aedes aegypti salivary peptide (the Nterm-34kDa peptide) and its potential use in measuring exposure to Aedes aegypti bites. Methodology/principal findings: A longitudinal study, concerning children (n=205, aged from 0 to 5 years) living in an area where Ae. aegypti is endemic in southern Benin (West Africa), was performed between 2008 and 2009. For each child, the specific immune response against the Nterm-34kDa peptide was evaluated by ELISA method, from a dried blood spot on filter paper collected every 6 weeks. The results demonstrated the existence of a specific IgG Ab response to Nterm-34kDa peptide in children exposed to Aedes aegypti bites. This response increased significantly for each year, from the dry season (low exposure) to the rainy season (high exposure). In addition the IgG Ab level was associated to the intensity of rainfall and presented spatial heterogeneity between individuals and between the studied villages. Conclusions/Significance: This preliminary study showed that IgG Ab responses to the candidate Nterm-34 kDa could be a promising alternative for evaluating the level of exposure to Aedes vectors and then the potential risk for getting arboviruses. It will be essential to validate this immuno-epidemiological tool by conducting further studies taking into account entomological measurements.

Keywords:*Antibody response, saliva, biomarker, exposure, Aedes aegypti, human arboviruses*

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O701M01

Entomology: General

O701

Preliminary study on Ketosynthase gene in *Paederus* Fabricius

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The degenerate primers of Ketosynthase (KS) gene were designed and in total 11 species of Paederus were amplified. Only females of P. fuscipes from 4 different collections were amplified with a fragment of about 1000 bp, named pedKS gene. pedKS gene is homologous with the KS gene of Pseudomonas to 76%-86%. NJ phylogenetic tree of representative KS gene from NCBI shows that pedKS gene possesses a close phylogenetic relationship with FAD-dependent oxidoreductase gene. Homologous gene in Pseudomonas fluorescens with pedKS synthesizes pyridine six membered rings by redox reaction. It is predicted that pedKS may also participate in the synthesis of pyran six rings in Pederin by oxidation reduction.

Keywords:*Paederus Fabricius, Ketosynthase, Pyran six rings*

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O701M02

Entomology: General

O701

Synchrotron X-ray microtomography and digital 3D imaging for examining insect morphology

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Due to their exoskeleton, internal morphological features of insects are often difficult to examine by traditional methods like light and electron microscopy. As synchrotron X-ray microtomography (SR-µCT) allows non-destructive imaging of millimeter-sized objects, it became a powerful tool for entomologists and was already considered a "methodological milestone in micro-anatomical research" (Betz et al. 2007). High-speed SR-µCT reduces the duration of a full tomographic scan to just a few seconds. Apart from dried samples, even ethanol-preserved specimens can be processed inside their containers without causing the fluid to boil. To analyze the image stacks, it is crucial to decide what visualization method is suitable to approach a particular scientific question. Volumetric data can be visualized by voxel-based volume renderings or by polygon surfaces based on labeled structures inside the image stack. Volume renderings are very suitable to quickly visualize objects as a whole. Further, virtual slices of selectable thicknesses allow examination of internal structural details without losing the 3D impression, as exemplified here for the head of the stick insect Peruphasma schultei (Fig.). While the creation of polygon surface models is time-consuming, it allows moving distinct body parts of a specimen with respect to each other. This method was used to interactively analyze and visualize the three-dimensional movements of the coxa-trochanteral joints of the weevil Trigonopterus oblongus, which constitute biological screw-and-nut systems.

***Keywords**:insect morphology, synchrotron X-ray microtomography, 3D imaging, volume rendering, virtual slice, polygon surface model*

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O701M03

Entomology: General

O701

Origin of the luminescence system in firefly

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Bioluminescence is one of the most fascinating phenomena in insects. The luminescence mechanism, luciferin-luciferase reaction, has been intensively studied using fireflies (Lampyridae). In contrast, the evolutionary aspects of the luminescence system remain obscure. I have studied on the origin of firefly luciferase and found that: (1) firefly luciferase arose from an enzyme involving in lipid metabolic pathway; (2) then, gene duplication gave rise to functionally different paralogues; (3) one of which is used for the luminescence in adult and another is for egg.

Keywords:*Firefly, Lampyridae, Coleoptera, Bioluminescence, Luciferase*

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O701M04

Entomology: General

O701

Formation of germ band in Diplura, with special reference to the differentiation of germ layers (Hexapoda)

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Diplura are included in Entognatha together with Protura and Collembola. However, some doubts have been cast on the monophyly of Entognatha, and recently a sister group relationship between Diplura and Ectognatha was suggested from various disciplines. Thus, Diplura are one of the most significant members in understanding the groundplan and reconstructing the phylogeny of hexapod basal clades. Uzel (1898) described the germ band formation in a rhabduran dipluran Campodea staphylinus Westwood. It is absolutely unique not only among Hexapoda but among Arthropoda, accompanied by peculiar manner of germ layer segregation, and the re-examination of the germ band formation in Diplura had been desired for long time (Jura, 1972). Ikeda (2001) light- and electron-microscopically observed the germ band formation in another rhabduran dipluran Lepidocampa weberi Oudemans, and reconfirmed the Uzel's observation. However, the information on dipluran germ band formation is still scanty and fragmented, and in addition our knowledge on it concerns only the suborder Rhabdura, that for another dipluran suborder Dicellurata being totally lacking. Aiming at the comprehensive understanding of the germ band formation in Diplura, we have been studying that in a dicelluratan Occasjapyx japonicus (Enderlein), also referring to the embryogenesis of a rhabduran L. weberi.

Keywords:*Diplura, mesoderm, endoderm, comparative embryology, germ layer*

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O701M05

Entomology: General

O701

The spruce budworm genome takes flight: Current endeavours and direction in sequencing the *Choristoneura fumiferana* genome

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The spruce budworm (Choristoneura fumiferana) (Lepidoptera: Tortricidae) is North America's most devastating defoliator of conifer trees and a model organism for the study of insect outbreak dynamics. This species is also part of a larger species complex, where the species are largely associated with different geographic ranges and host plants, allowing the group as a whole to be fascinating in the study of adaptation and speciation. Sequencing and assembling the C. fumiferana genome will provide researchers with a much needed tool to further study this group of forest insect pests. Our sequencing strategy combines several NGS technologies including: (i) Roche 454 GS-FLX and GS-FLX+ shotgun sequencing, (ii) GAIIx Illumina sequencing of a 2 kb paired-end library, (iii) 454 GS-FLX sequencing of 6 kb and 20 kb paired-end libraries, and (iv) 454 GS-FLX+ sequencing of pools of 50 BAC clones (average insert size of 140 kb). For identification of expressed ORFs during the annotation process, we have also conducted 454 GS-FLX+ sequencing of a normalized cDNA library developed from a pool of RNAs extracted from all budworm life stages. These transcriptomics resources complement existing EST libraries developed by our group. Several companion studies include Illumina sequencing and mapping of other Choristoneura species, inter- and intraspecific genotyping-by-sequencing studies, and tissue-specific RNA-seq transcriptomics. Here, we will report our progress to date in assembling and annotating the genome, and further describe the companion studies that are currently underway.

Keywords:*Choristoneura fumiferana, spruce budworm, genome*

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O701M06

Entomology: General O701

Entomotoxic and membrane-disturbing properties of urease and derived peptides

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Ureases, enzymes that catalyze the hydrolysis of urea into ammonia and CO2, also display biological properties unrelated to ureolysis, such as fungitoxic and insecticidal activities. Plant ureases are lethal to insects not susceptible to Bt toxins. In the case of jackbean urease (JBU), insecticidal activity relies mostly on an internal peptide (jaburetox-2) released by the insect's digestive enzymes. However, whole ureases show entomotoxic properties per se. Here urease and derived peptides were tested for membrane-disturbing properties using Planar Lipid Bilayers (PLB) and the carboxyfluorescein release assays. Aiming to establish structure X activity relationships, Jaburetox-2 mutants were obtained: deletion of aminoacids 61-75 (deltaBeta-hairpin); deletion of N-terminal (delta1-44) and of the C-terminal (delta45-92) halves. JBU and Jaburetox-2 (5-10 nM) inserted into the PLB forming ionic channels. JBU's channels displayed conductance levels of 1730, 833, 625 and 352 pS, with apparent anion selectivity. Jaburetox-2 also forms channels with different conductance and kinetics. Jaburetox-2 (72 nM) induced leakage of carboxyfluorescein from large unilamellar vesicles composed of acidic lipids. The deltaBeta-hairpin mutant showed all the properties of wild jaburetox-2, indicating that the Beta-hairpin motif is not relevant for its membrane-disturbing ability. The halves of jaburetox-2 molecule (delta1-44 and delta45-92) showed much decreased activity on PLB and carboxyfluorescein leakage assays, suggesting the presence of an active domain that was "split" between these two mutants. Membrane-disturbing and ion channel forming activities of urease and derived peptides may contribute to their diverse biological activities, as plant defense against insects and pathogens. Financial support: CAPES, CNPQ, FAPERGS, SEVE Centre.

Keywords:*urease, jaburetox, insecticide, membrane-disturbing, pore-forming, ion channel property*

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O701M07

Entomology: General O701

Differences in egg morphology between coleopteran and lepidopteran stored-product insect pests in relation to tolerance to fumigants

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Eggs of Coleoptera are more tolerant to fumigants than those of Lepidoptera. Driedfruit beetle (DFB, Carpophilus hemipterus, Coleoptera: Nitidulidae) and navel orangeworm (NOW, Amyelois transitella, Lepidoptera: Pyralidae) are endemic to California and pose a threat to the California dried fruit and nut industry. Post-harvest chamber fumigation is a critical element of the DFB and NOW pest management strategy. However, eggs of DFB are difficult to kill using sulfuryl fluoride. Therefore, we studied the external morphology of DFB and NOW eggs using a scanning electron microscope to identify any structural differences that may explain their differential response to sulfuryl fluoride. We found that aeropyles, which enable gaseous exchange in eggs, are few (2) in DFB and restricted to only one end of the egg. On the other hand, NOW has a greater number of aeropyles (7) distributed on both ends of the egg. The micropyle, which is an opening for sperm entry that also facilitates gaseous exchange, is absent in DFB but present in NOW. It is possible NOW eggs with a micropyle and several aeropyles may have a higher rate of fumigant penetration than DFB. In DFB eggs with only 2 aeropyles, the route for fumigant entry may predominantly be diffusion through the chorion. However, fumigant entry into the egg by diffusion through the chorion may be comparatively slower compared to entry through aeropyles and/or micropyle. Our findings provide a possible explanation for why coleopteran eggs are more tolerant to fumigants than lepidopteran eggs.

Keywords:*insect eggs, tolreance, aeropyles, driedfruit beetle, navel orangeworm, fumigants*

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O701M08

Entomology: General

O701

Analysis of a chromosomal region harboring the sex determination locus in the yellow fever mosquito *Aedes aegypti*

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Aedes aegypti is a major vector of viruses infecting up to 100 million people annually. Genetic approaches to Aedes control hold promise for a successful management of these diseases, but creation of the necessary tools depends on the knowledge of developmentally-regulated sex-specific mosquito genes. We constructed an A. aegypti strain (C42), in which a single transgene insertion occurred within a repetitive sequence tightly linked with the maleness locus on chromosome 1. As a result, males from this strain possess an eye promoter-controlled EGFP tag. However, rare crossing-over events during male meiosis break the linkage between the maleness locus and the transgene. We established two recombinant lines (A and B), in which females carry the EGFP-positive chromosome 1. Females of line A crossed to any male produce progeny with a distorted sex ratio (1♂:2♀), because all F1 embryo males inheriting the maternal recombinant chromosome 1 die. Moreover, crosses involving double recombinant females that secondarily lost EGFP during crossing-over yield progeny with an inverted sex ratio (2♂:1♀), also caused by embryonic lethality. Line B females produce progeny with a Mendelian inheritance pattern; however, inbreeding the EGFP-inheriting progeny leads to homozygote females that die during emergence from pupae. These results indicate the existence within the recombination region of genes vital for sex-specific development, whose location safeguards against homogenization of gender-specific sex determining loci. The described lines represent a unique resource to molecularly identify all the loci involved.

Keywords:*sex determination, sex-specific lethality*

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O701M09

Entomology: General

O701

Egg structure and embryonic development of *Eucorydia yasumatsui* Asahina (Blattodea, Polyphagidae)

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Although an assemblage of the Blattaria, Mantodea and Isoptera or the Dictyoptera is well supported to be monophyletic, the systematic relationships between three ordinal constituents have not been settled, despite a lot of morphological and molecular phylogenetic studies, besides, which often bestow a paraphyletic status to Blattaria. Therefore, for the reconstruction of groundplan of Dictyoptera and their phylogeny, the understanding of Blattaria is the most significant. For the solution of phylogenetic problems, the comparative embryological approach is promising. Many embryological studies have been made on Blattaria. However, they concern only the species well known as pests of the derived families Blattellidae (Blattella germanica) and Blattidae (Periplaneta spp., Blatta orientalis), and it is difficult to figure out the embryological groundplan of Blattaria. In such a background, we have planned a comparative embryological study covering the representative members of cockroaches from primitive to advanced. As the first step of our study, we tackled with Polyphagidae, which is one of the proposed basalmost clades in Blattaria, using a Japanese polyphagid Eucorydia yasumatsui Asahina. We show the structural features of its eggs and provide an outline of its embryonic development. The eggs are provided with several micropyles on their posterior ventral side. The embryo forms at the ventral side of the egg, and develops there without changing its original position and axis, as in the embryos of Blattellidae. A drastic changing of embryo's axis as shown in the embryos of Periplaneta and Blatta is not involved in the blastokinesis of E. yasumatsui.

Keywords:*Dictyoptera, Blattaria, Polyphagidae, comparative embryological approach*

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O702TU01

Entomology: General

O702

Weevil evolution and differential gene expression in the development of the rostrum

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Weevils (Coleoptera: Curculionoidea) are a diverse group of extant organisms with approximately 50,000 described species. They are of great agricultural significance because they are associated with all major groups of plants and plant tissues. The weevil rostrum, for example, is a key evolutionary innovation that has enabled this group to feed on and oviposit in nearly all plant tissues, giving rise to diverse life histories and tremendous diversity in rostrum form. Insights into comparative development of the rostrum will provide insight into the evolution of this key innovation that may be responsible for the explosive radiation of the lineage. Although weevils are an enormous group and countless species are significant agricultural pests, no weevil species have been utilized in developmental studies. In order to better understand the formation and evolution of this structure, histological examinations have been made in a phylogenetic context for comparing the internal structure of the rostrum in adults. Transcriptomes from the developing head tissue of 4 weevil species, representing disparate clades and divergent rostral forms, and 1 outgroup (non-weevil species) have also been produced in de novo assemblies. While there are difficulties in assessing differences among transcriptomes from divergent taxa, tests for differential expression patterns of transcripts are being performed to characterize differences in the gene networks that are producing the profound phenotypic diversity observed in the rostrum and to better understand the genetic framework that permitted the diversification of such an immense lineage as the weevils.

Keywords:*weevils, gene expression, phylogeny*

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O702TU02

Entomology: General

O702

Microsatellite markers in the era of Next-Generation DNA sequencing: Informative and cheap genetic markers for entomological research

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Microsatellites are among the most frequently used DNA markers in many areas of research, including entomology. These genetic markers can be developed for non-model species and are highly informative when investigating genetics and dynamics of insect populations: genetic structure of populations, estimation of gene flow between taxa, patterns of fine-scale dispersion, determination of pest insect invasion routes, etc. This presentation first summarizes the main advantages of microsatellite markers and gives several examples of their use in entomology. Second, it focuses on the new perspectives open by Next Generation DNA sequencing that facilitates the setup and use of microsatellite markers. In particular, the results of the "EcoMicro" project carried out by a large consortium of European teams will be presented. This project proposed a revised isolation method based on the 454 GS-FLX Titanium pyrosequencing of microsatellite-enriched DNA libraries. This procedure, that has been tested on >100 species, makes microsatellites more and more accessible to entomologists. New developments of genotyping methods based on Next-Generation sequencing are also expected to increase the cost-efficiency and informativeness of microsatellite markers.

Keywords:*SSR, multiplexing, population genetics*

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Genetic structure of the mealybug *Pseudococcus viburni* (Hemiptera: Pseudococcidae) populations worldwide.

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Pseudococcus viburni is a mealybug pest species attacking more than 46 families of plants, including important agricultural crops, worldwide. This widely spread species also displays remarkable morphological variations at diagnosis characters and DNA variations at nuclear and mitochondrial genes, suggesting the presence of differentiated population clusters. Here we used microsatellite markers to investigate the genetic structure of P. viburni populations worldwide. Microsatellite markers were developed from a pyrosequenced microsatellite-enriched DNA library. Twenty two multiplexed markers were then used to genotype twenty populations with different geographical origins. Population clustering analyses and genetic differentiation estimates were computed from the data. The results reveal low genetic variability within populations (heterozygosity rates ranging from 0.127 to 0.438 and an average number of alleles per population ranging from 0.739 to 5.826). They also reveal high Fst values between populations, ranging from 0 to 0.679 (mean Fst of 0.35). Clustering analyses suggest the occurrence of two population groups, one composed of South American populations, the other composed of other populations, while a population from New Zealand displays individuals from the two groups. Based on these results and those obtained with mitochondrial and nuclear ribosomal DNA, we discuss the factors impacting the population genetic structure.

Keywords:*Mealybug, Pseudococcus viburni, genetic structure, microsatellites*

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O702TU04

Entomology: General

O702

Sex determination genes and *Wolbachia* in Queensland fruit fly, *Bactrocera tryoni*

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Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) is Australia's most destructive horticultural pest. Suppression methods, including the release of irradiated, sterile individuals or individuals infected by Wolbachia (α-Proteobacteria), are techniques that will become increasingly useful with the introduction of a male-only strain. Wolbachia are intracellular bacteria of insects that can cause crossing incompatibilities and may interfere with genes involved in sex determination. To ultimately achieve a male-only strain of B. tryoni, a thorough understanding of the genes that form the sex determination pathway is necessary. The pathway has been well studied in Drosophila melanogaster (Meigen) (Diptera: Drosophilidae), and this has provided a starting point for characterising genes involved in sex determination in other Diptera. However, the initial signal in male Bactrocera is communicated by the Y-located dominant male determiner, M, which is absent in Drosophila species. The mode of action of M, and its direct targets have yet to be characterised in fruit flies or in any other Y-determined insect. A Y chromosome marker carried in a B.tryoni line can distinguish male and female embryos with a simple PCR assay, permitting the use of quantitative PCR to study expression of important sex-determination genes in sexed single embryos. This has helped to clarify the timing of M, and will enable transcriptome sequencing of separated male and female embryos at targeted developmental times.

Keywords:*Bactrocera*

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O702TU05

Entomology: General

O702

Clearance of an errantivirus sequence in the production process of trivalent recombinant hemagglutinin (rHA) influenza vaccine, FluBlok®

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Errantivirus is a group of invertebrate retroviruses, including an exogenous virus and LTR-retrotransposon based on ICTV classification. Dm gypsy virus is the only identified exogenous errantivirus. Exogenous errantiviruses have not been reported in lepidopteran insect cells, which are extensively used in recombinant protein production in the biotechnology and pharmaceutical industry. However, a low level of reverse transcriptase (RT) activity has been reported for lepidopteran cell substrates, raising questions whether this activity might particle-associated. Recently, a 240bp sequence similar to conserved domain of pol gene of errantivirus was cloned from genomic DNA in Sf9 and High5 cells. We also identified and cloned a 240bp sequence, Sf-37, from genomic DNA of our expresSF+® (SF+) cells and confirmed expression by RT-PCR analyses. We developed an RT-PCR for in process sample testing and an even more sensitive nested PCR method to address concerns of whether this Sf-37 sequence is present in our drug product, FluBlok®. We used a RT-PCR method to screen for the presence of Sf-37 RNA signal at various stages in the purification process and demonstrated that the Sf-37 RNA sequence is removed during the early up-stream purification process of our drug product. FluBlok® vaccine was concentrated by ultracentrifugation, and subsequently tested in the RT-PCR follow by the nested PCR. The nested PCR method that can detect a single copy of RNA was used for drug product testing. We confirmed that the Sf-37 RNA sequence cannot be detected in three doses of FluBlok®. This work was funded in part under contract HHSO100200900106C.

Keywords:*errantivirus, retrovirus, reverse transcriptase, Spodoptera frugiperda cell, PCR*

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Growth performance evaluation and productivity of *Bombyx mori*(L) (Silkworm) fed with three varieties of *Morus alba*(L) mulberry plant

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Ogunleye, R.F. and Popoola, D. O Department of Zoology, Ekiti State University, Ado Ekiti E-Mail: droluwaleye@yahoo.com Seunpop34@yahoo.com ABSTRACT The research work was carried out to evaluate and compare the growth performance and productivity of a strain of silkworm, Bombyx mori (EC1) fed with three different varieties of mulberry plant (K2, S-36 and E-Lobe).The experiment was carried out at ambient conditions 37oC and 55% R.H. The parameters used to evaluate the growth performance were larval length, width and weight while the characters used for productivity include shell weight, cocoon weight, shell ratio , filament weight , length, denir and number of breaks. The result revealed that the performance of mulberry leaves varied from each other and that silkworms fed with S-36 variety performed significantly higher in terms of growth and productivity. The next variety to it is the K2 and followed by E-lobe variety.

Keywords:*Silkworm, growth, performance, mulberry variety*

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O702TU07

Entomology: General

O702

Ultrastructure of the antennal sensory organs of two *Fannia* species

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Fannia (Muscoidea: Fanniidae) species are the most important mechanical vectors of faecal-borne pathogens, larvae of them can cause intestinal and genital myiasis in men or domestic animals. Currently, there is a paucity of morphological information on antennal sensory organs of Fannia. To fill in the gap, we investigated the ultrastructure of antennal sensilla in two ubiquitous Fannia species (F. canicularis and F. scalaris) using scanning electron microscopy. Four distinct types of sensilla, including trichoid, basiconic, coeloconsa and clavate sensilla, and one type of olfactory pit are recorded in F. canicularis and F. scalaris. A cross-sectional view of the funiculus shows it to be triangular, with an anterodorsal surface, a dorsolateral margin and a posteroventral surface. The latter presents one large-size pit, containing basiconic sensilla, on median areas of the distal funiculus. Type, number, density and distribution of antennal sensilla and olfactory pit in Fannia species are compared with other muscoid flies : phytophagous anthomyiids and saprophagous muscids. According to the results above, the evolutionary trends and the function of antennal sensory organs are discussed in Muscoidea. Therefore, such studies would enrich the information about antennal sensilla of Fanniids, and contribute to the development of synthetic attractants for the monitoring and control of medical fly populations.

Keywords:*Fannia, antenna, sensory organ, scanning electron microscopy*

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O702TU08

Entomology: General

O702

The molecular genetics of the oily (translucent) mutants in the silkworm, *Bombyx mori*

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Uric acid is the end product of nitrogen excretion in insects. It is also accumulated in tissues and organs such as fat body, epidermis and wing. The silkworm, Bombyx mori, is a typical insect accumulating uric acid in the body, and regarded as a model insect for the study on uric acid metabolism in insects. Uric acid is accumulated as urate granules in the epidermis of the silkworm, which makes the larval skin color white and non-translucent. More than 35 'oily' mutants of the silkworm show the translucent larval skin due to the absence and insufficiency of urate granules, and accordingly their larval skin color is more or less translucent. The genes controlling the oily phenotypes are dispersed among different loci on various chromosomes. Recently, some of them have been identified at the nucleotide sequence level. The oq and og mutants have the highly translucent larval skin due to the lack of xanthine dehydrogenase activity. The ow and od mutants are caused by the mutations of the genes encoding the proteins required for intracellular membrane traffic. The w-3 and os mutants are caused by the deficiencies of membrane transporter proteins. In our study, we found that the repression of dysbindin in the ov mutant possibly causes the translucent skin. The ok mutant is resulted from the abnormality of an ABC transporter. The study on oily mutants would improve our understanding of uric acid metabolism in insects and other organisms.

Keywords:*Bombyx mori , translucent larval skin , uric acid*

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O702TU09

Entomology: General

O702

Assembly of empty Israeli acute paralysis virus capsids in baculovirus infected insect cells

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The dicistrovirus Israeli Acute Paralysis Virus (IAPV) has been implicated in the worldwide honey bee decline linked to colony collapse disorder (CCD). Studies of the virus in pure culture would be valuable but are restricted by available isolates and permissive cell culture. Here we show that coupling the IAPV major structural precursor protein ORF2 to its cognate 3C-like processing enzyme results in processing of the precursor to the individual structural proteins following expression by a recombinant baculovirus in a number of insect cell lines and that the efficiency of expression is influenced by the level of 3C protein. The mature IAPV structural proteins assembled into empty capsids that were identified by their sedimentation profile in sucrose velocity gradients and peak fractions showed typical dicistrovirus like particles when examined by electron microscopy. Capsids reacted strongly with a polyvalent serum generated to a fragment of ORF2 expressed in bacteria suggesting the feasibility of a simple diagnostic for the presence of IAPV infection. Recombinant IAPV capsids may be useful for on-going investigation of the causes of CCD and similar expression technology may allow defined reagents to be generated for many other bee viruses within the picornavirus family.

Keywords:*IAPV, honeybee, colony collapse, baculovirus, diagnostics*

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O703F01

Entomology: General

O703

Olfaction in a vector fruitfly *Phortica variegata*

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The primitive fruitfly, Phortica variegata belonging to the family of Drosophilidae and to the Steganinae subfamily, shows a peculiar and rather unusual zoophilic behavior unlike other fruitflies of the same family. Apart from feeding on fruits, P. variegata flies also feed on lachrymal secretions of animals and humans thus transmitting Thelazia callipaeda (Spirurida, Thelazidae) eyeworms that causes thelaziosis, potentially leading to blindness. What contributes to this special zoophilic behavior in these flies remains unknown. Since olfaction plays an important role in the life of other pathogen transmitting vector insects, we investigated the olfactory system of these flies to understand its role in their distinct zoophilic behavior. In this study, for the first time, we describe the olfactory system of a vector fruitfly. We show that P. variegata flies have well-developed olfactory system similar to their well-known counterpart Drosophila melanogaster. In P. variegata, the peripheral olfactory organs, the antenna and maxillary palp, harbor distinct types of olfactory sensilla. The primary olfactory center in the brain, the antennal lobes, shows a glomerular organization and receives neuronal input from the peripheral olfactory organs in an organotopic pattern. Further, using electrophysiological methods we demonstrate the ability of these flies to detect both fruit related and animal related odors. These results allow us to compare and discuss the olfactory system of P. variegata with that of the well described D. melanogaster olfactory system.

Keywords:*Olfaction, Drosophila, Phortica variegata, vector biology, thelaziosis, eye worm*

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O703F02

Entomology: General

O703

Sexual conflict among the lichen tuft moths.

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Insect species display extraordinary variation in male genital morphology; therefore genital evolution is rapid and divergent. Previous assumptions for diversification in genital morphology were based on species isolation and pleiotropy, but subsequent comparative research has failed to support these hypotheses. Recent comparative and experimental research has proposed sexual selection to be the most likely driver of rapid and divergent evolution of genitalia, either via sperm competition, cryptic female choice, or sexual conflict. Sexual conflict may drive genital evolution through opposing selection of male and female reproductive strategies as genital adaptations that allow males a competitive advantage may consequently reduce female fitness leading to an evolutionary arms race via sexually antagonistic co-evolution (SAC). Lichen tuft moths of the genus Izatha (Lepidoptera: Oecophoridae) are excellent candidates for exploring the potential for sexual conflict to drive genital evolution, as some males have detachable spines (deciduous cornuti) which are ejected into the female reproductive tract during their first mating, apparently causing damage. Some species lack deciduous cornuti, but have permanently attached sclerotised teeth on the phallus, whilst others lack these structures all together. Here I present a molecular and morphological phylogenetic analysis of the genus that provides insight into the evolution of these complex genitalic adaptations. Genital complexity appears to have evolved along multiple paths, via the modification of different genital structures. Izatha therefore may represent an ideal model for the study of genital evolution.

Keywords:*Reproductive strategies*

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O703F03

Entomology: General O703

Interactions between native insects and Australian invasive acacias in Europe

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Over 20 species of Australian acacias have been introduced in Europe, most of which became invasive and threaten the sustainability of native ecosystems. However, interactions between exotic acacias and the native entomofauna remain largely unstudied. Further, the role of exotic introduced insects involved in links of the food webs of native ecosystems, is also unknown. Research was conducted in Portugal, on Acacia longifolia and Acacia saligna, two of the most problematic invasive arboreal species in southern Europe. The composition of the communities of potential insect pollinators, associated with the acacias, was studied and comparisons made between the flowering and post-flowering periods. Although significantly more Hymenoptera and Coleoptera were caught when inflorescences were present, thus indicating that generalist pollinators likely promote seed production, their contribution to the invasive potential of A. longifolia should remain low. The role of ants in the dispersal of A. saligna seeds was also investigated. It was concluded that some common native ant species, particularly Aphaenogaster senilis and Messor barbarus, are efficient seed dispersers and promote the establishment of seed banks. Since acacia seeds may remain viable for decades, granivorous ants likely play an important role in their establishment and spread. By contrast, the exotic Argentine ant, Linepithema humile, that has invaded the coastal areas of Portugal, consumes the eliosomes of A. saligna seeds but do not translocate them, thus decreasing their fitness. Findings are innovative and uncover mutualistic relationships, presently evolving between native insects and exotic plants, which result in an adaptative advantage for the invaders.

Keywords:*invasive species, mutualistic interactions, native entomofauna*

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O703F04

Entomology: General O703

Enhancement of fruit quality in green pepper through pollination by *Hypotrigona gribodoi* in small scale farms in western Kenya

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A study was carried out in Kakamega forest, western region of Kenya, to evaluate the effectiveness of the stingless bee Hypotrigona gribodoi Magretti on pollination of green pepper (Capsicum annuum L.). Three treatments were applied and consisted of self-pollination, pollination by feral pollinators in open field and pollination by H. gribodoi in net cage. The difference of yield in fruits and seeds quality was compare among the treatments. Plants whose flowers were pollinated by H. gribodoi produced heavier fruits with a higher number of seeds compared to self-pollinated flowers or flowers pollinated by feral insects. Moreover, seeds were significantly bigger in size in fruits resulting from flowers pollinated by H. gribodoi compared to fruits obtained from self-pollinated flowers or flowers pollinated by feral insects. We therefore conclude that H. gribodoi is an efficient pollinator of green pepper in tropical region of East Africa.

Keywords:*Hypotrigona gribodoi, green pepper flowers, fruit and seed quality*

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O703F05

Entomology: General O703

Research and utilization of Chinese gall aphids (Hemiptera: Aphididae: Fordini) in China

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Chinese gall aphids are a small group among the gall-inducing aphids. They include six genera, ten species, and four subspecies which have complex life cycles with cyclical parthenogenesis and multiple generations with alternative hosts. Their life cycle includes sexual and asexual reproduction, and alternation between the primary hosts (Anacardiaceae: Rhus) and certain secondary hosts (mosses). Three primary host plants and more than fifty secondary host mosses have been reported in China. Each gall aphid species or subspecies form a characteritic, species-specific gall on its primary host. The galls, which called Chinese gallnuts (Wubeizi), are sealed and sac-like which show great variation in shape, size, structure, and galling-site. Generally, each gall is initiated by a single fundatrix which reproduces parthenogenetically for three generations within a gall and the apterous fundatrigeniae live in it for about three to five months. The most prominent species is Schlechtendalia chinensis, which forms horned-like galls on Rhus chinensis. The gallnuts have been mainly used for medicine and chemical purposes for more than two thousand years because they are comprised mainly of tannins. They are still important traditional resources and commercial exports in China until now. The annual yield of gallnuts in China is 5-6 million kilograms and about 95% of the total world yield. Many efforts have been made to study the artifical breeding techniques of aphid in order to increase the yield. It is a main income source for the farmers who rear and utilize the gallnuts in Southwest China.

Keywords:*Chinese gall aphid, gallnut, tannins, medicine, aphid artifical breeding.*

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O703F06

Entomology: General

O703

Evolution and biogeography of *Bombyx mandarina* compared with the domesticated mori revealed by Bayesian inference of DNA sequences data from some transposons including mariner-like elements (MLEs)

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Ancestral inferences based on polymorphism data have been great interests in biogeographical studies using natural populations. And for taking advantage of the transposons including mariner-like elements (MLEs) as an evolutionary clock, reliable estimation for the time of each event is required. In this presentation, we demonstrate our coalescent approach is useful tool to estimate times of evolutionary events within a species and the ancient mariner-like element (MLE) sequences can be applied as a molecular marker to understand geographical relationships among distantly related species. "BmTNML" locus is composed of three different transposons (Bmmar2 type of MLE, L1Bm and BMC1 retrotransposable elements) in almost all the mori strains (Nakajima et al., J. Mol. Evol., 1999). And it is recently cleared that this unit exists only one copy on the Chromosome 6 of B. mori. But the transposons insertion patterns in this locus of mandarina are very different from those of mori. Based on the genealogy defined by insertions/deletions of the transposons in this locus, we estimated the most common recent ancestors (TMRCAs) and ages of these events by using the 45 sequences of the TEs and host genome from mori and mandarina. These results suggested the neutrality of the MLE insertion in the B. mandarina population and the MLE inserted into the common ancestor's genome of mori and mandarina. It was also strongly supported that the new discovered maT type of TEs insertion and the omission event in this locus might be occurred very recently only in the restricted population of mandarina.

Keywords:*mariner-like element, L1Bm, BMC1, Bm1, BmamaT1, coalescent model, TMRCA*

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O703F07

Entomology: General O703

Queen selection in three genera of social wasps of tribe Epiponini (Vespidae: Polistinae)

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Queen selection is a very complex process, and it is poorly studied in most of species of neotropical swarm-founding wasps. Queen selection was described in detail only for Metapolybia aztecoides. Since then, few studies were performed to understand how queens are selected. Queen selection could vary between species because there is a lot of variation across the tribe. For this reason, we studied queen selection process in three genera of Epiponini in different stages of colony cycle. Two colonies of Metapolybia, Chartergellus and one of Protopolybia were observed in Brazil and Costa Rica. To identify queens, eggs were removed from cells to stimulate oviposition. Egg-laying females were marked. Queen behavior and their interactions were observed. When colonies were not in a queen selection stage, queen removal tests were performed to stimulate new queens production and to observe behavioral responses in orphan colonies. There was an increase in the amount of display behaviors during queen's selection stage. After queen elimination, the amount of egg-laying females increased, mainly in Metapolybia and Chartergellus. In most colonies new queens were old females with less developed ovaries. Our results showed that females from these social wasps are reproductive plastic, that even old females could develop their ovaries in the absence of queens. In consequence, workers can recognize and respond to queens' absence. Also, we show important differences in queen selection process between genera, which also could be related to colony cycle.

Keywords:*queen selection, social interactions*

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O703F08

Entomology: General

O703

ButterflyIndia: Cyber community initiative to document Indian butterflies

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Internet has become integral part of our daily life in past few years and is changing many of the things we do on day to day basis. Biodiversity documentation and citizen science are positively impacted too. Here we present a case study of a cyber-community of Indian Butterfly lovers and how it is harnessing the internet in its operations. Initiated in 2001 with mere 25 members, it has grown to more than 1450 member and has exchanged more than 25000 messages. The group has organised 12 meets which are a combination of field-trips and mini-conferences. The group has contributed to numerous books, publications and websites documenting butterfly diversity of Indian subcontinent. There have been several discoveries of rare and very rare butterflies by citizen scientists and confirmations by experts through the e-group. The whole group works on no profit no loss basis.

Keywords:*ButterflyIndia, Butterflies, India, Internet*

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Progress of eriophyoid mite research in China (Acari: Eriophyoidea)

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The eriophyoid mites, unlike other Acari, have only two pairs of legs. Body fusiform or vermiform in shape, white or light yellow in color, usually 200μm in length and 70μm in width. Normally, eriophyoid mites cannot be found by naked eyes. Eriophyoid mites are obligate plant feeders with strong host specificity. Some species have great economic importance in agriculture, such as Aculops lycopersici (Tryon, 1917) infesting Tomato, Phyllocoptruta oleivora (Ashmead, 1879) infesting Citrus sp. (Rutaceae), Aceria tosichella Keifer, 1969 infesting Wheat and Barley and can transmit virus (WSMV). Up to 2011, there are three families, 357 genera and 4,404 species reported in the world. Up to 2010, 932 species belonging to 3 families and 187 genera are reported in China. The taxonomy of eriophyoid mites in mainland China has developed rapidly in recent years. Trotter (1900) is the first person describing new eriophyoid mites collected in China. He reported two new species. Hai-Yuan Kuang (1980) is the first Chinese who conducted taxonomic research of the Eriophyoidea in mainland China. During 1980 to 2005, Kuang and his co-authors described 244 new species from China. Xiao-Yue Hong and his co-authors described 232 new species from China, and most of the species were described from 2005 to 2009. Sui-Gai Wei and his co-authors described 122 new species from Guangxi Zhuang Autonomous Region in China. Kun-Wei Huang and his co-authors described 229 new species from Taiwan. Biology and control of eriophyoid mites have been focused only on a few species.

Keywords:*eriophyoid mites, Eriophyoidea, Acari*

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O703F10

Entomology: General

O703

Undergraduates gain experiential learning experience through unique pollination biology research program

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Research opportunities provide undergraduate students with valuable experiential learning experiences that enhance their degree programs, and enable them to be competitive for graduate school admission and in the job market. In particular, focused research training provided to a cohort of students on a topic of current relevance enables training of a new generation of scientists with the ability to address global issues. However, universities offer little, if any, support for development of such programs. At Oregon State University, we hosted a National Science Foundation supported REU (Research Experiences for Undergraduates) Site program that trained undergraduates in comparative, multidisciplinary research in pollination biology over 10 weeks in the summer. Students were recruited nationwide, and provided background information on bees, plants, ecology, conservation, sampling and statistical analyses. They examined plant-pollinator interactions while rotating through three ecologically distinct sites across Oregon for experience with both fundamental and applied approaches to pollination research. Initially, they helped with existing projects at all three sites. Subsequently, they conducted independent research on a specific topic and presented their results during a Pollination Conference. Program outcomes assessed through pre and post quizzes indicate enhanced knowledge about entomology, research methodology, and conservation issues. Feedback received 1-2 years later indicated that the program motivated alumni to seek opportunities to present and publish their REU experiences, to conduct senior thesis research related to bees and pollination, seek additional research experiences, and to enroll in graduate programs. The program also enabled them to receive highly competitive fellowships for national and international research.

Keywords:*Pollination biology, experential learning*

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O703F11

Entomology: General

O703

Biorational control of kaydid pests on oil palms in Papua New Guinea

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More than 80% of the reports of pest problems in Papua New Guinea each year derive from the Sexava group of katydids (Tettigoniidae). Current control relies on chemical treatment using methamidophos, which is unsustainable due to the high cost, its failure to significantly reduce loss, and the impending discontinuation of supply. We are investigating an alternative pest control approach using a fungal delivery system for insecticidal proteins to rapidly and effectively control katydid pests. In this study, isolates of the entomopathogenic fungus, Metarhizium anisopliae, were identified as potential biopesticide candidates to control katydid pests on oil palm crops in PNG. Tests were performed in the laboratory using a range of fungal concentrations on several insect species to determine the infectivity of the fungal isolates to long-horn grasshoppers and the specificity of the candidate fungal biopesticides. Since rapid mortality is needed to effectively control katydid damage, the results of this study will identify a fungal isolate(s) to be genetically engineered to express insecticidal peptides from scorpion or spider venoms. This will increase the virulence of the biopesticide agent, reducing the required spore dose and time of treatment.

Keywords:*Metarhizium anisopliae, Biocontrol, entomopathogenic fungus, Sexava, biopesticide*

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O703F12

Entomology: General O703

Prevention of incursion of coffee berry borer (*Hypothenemus hampei*) into Papua New Guinea and implementation of national surveillance scheme.

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Coffee Berry Borer (Hypothenemus hampei) (CBB) originates in Africa and has spread to the coffee growing regions of the world causing severe damage to coffee and affecting smallholder livelihoods. CBB bores into the distal end of cherries and produces progeny which form 'galleries' in the bean and this can frequently cause up to 80% crop yield loss. The spread of CBB is facilitated through movement of coffee cherries and gunny bags. In 2008, CBB was recorded 50km from the border of CBB-free Papua New Guinea, in Oksibil, Indonesia, threatening the livelihoods of PNG coffee farmers. Almost 50% of rural households in PNG are involved with the coffee industry. To pre-empt potential effect on livelihoods, a project investigating the prevention of incursion of CBB into PNG and to monitor potential pathways of entry and spread into and within the country was initiated. This ACIAR funded project (a collaboration between CABI, Coffee Industry Corporation of PNG and NAQIA - National Agricultural Quarantine Inspection Authority) aimed to train officials to recognise signs and symptoms of infestation, assess the movement of coffee (at the farm level and through trade), and to establish a national surveillance scheme to monitor for the pest internally to facilitate early detection. The work identified the importance of several potential vectors and pathways of movement for CBB, highlighting the risk of incursion and movement of CBB. An overview of the analysis of vectors and pathways and the surveillance methodology is presented.

Keywords:*Coffee Berry Borer, Hypothenemus hampei, Pathways,Papua New Guinea*

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O703F13

Entomology: General

O703

Efficient production of knockout crickets using ZFNs and TALENs

Takahito Watanabe[1], Hiroshi Ochiai[2], Tetsushi Sakuma[3], Taro Nakamura[4], Taro Mito[5], Takashi Yamamoto[6], Sumihare Noji[7]

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An orthopteran insect, the cricket Gryllus bimaculatus, has recently become a hemimetabolous insect model system, whereas target mutagenesis was intractable in this species as in other non-Drosophila systems. Here we use custom designed nucleases technology, zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN), in cricket embryos to introduce sequence-specific mutation via nonhomologous end joining (NHEJ)-mediated deletions or insertions at the target site. To examine the potential of custom designed nucleases in the cricket, we designed a ZFN pair for EGFP, and microinjected mRNA into fertilized eggs expressing EGFP in the cytoplasm. As a result, we obtained germ-line mutant crickets at the high rate (48%). Next, we attempted to induce mutations into an endogenous gene Gb'laccase2, which has been shown to be involved in cuticle tanning. We designed a ZFN and TALEN pair for Gb'laccase2, and microinjected mRNA of these nucleases into wild-type fertilized eggs. Spots of tanning defects were observed in nuclease-treated G0 nymphs, indicating somatic mutations were induced by ZFNs and TALENs. 47% (ZFNs) and 17% (TALENs) of founder animals transmitted disrupted gene alleles. Via selection of G1 heterozygotes by mutation detection assays and subsequent sibling crosses, we obtained homozygous targeted alleles exhibiting expected cuticle-tanning-defective phenotypes. Our approach depends neither on the particular mutant phenotype nor genetic tractability of the organism. It should be generally applicable to any genomic locus in organisms that allow mRNA delivery into the embryo. Thus, our results should facilitate the direct genomic manipulation of non-model organisms and their analysis by functional genomics.

Keywords:*cricket, ZFN, TALEN*

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O703F14

Entomology: General

O703

The change of fauna and soil characteristics for Saemankeum reclaimed region in Korea

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Saemankeum is the largest region, changed into reclaimed region in Korea, recently. The first purpose of Saemankeum reclaimed region is the development of new farmland. Since 2006, the construction of embankment, Saemankeum region has been isolated from the sea, slowly dried and changed into the land. The soil animal are very important for the formation of agricultural soil and also represent a large portion of the healthiness of agricultural soil. The soil animal carry out a wide range of ecosystem process that are essential crop production, soil resource quality and environmental health. In order to examine the change of reclaimed region related with the formation of new farmland, we examined the soil chemical properties and the emergence of the soil mesofauna in Saemankeum reclaimed region in 2006, 2011, separately. We investigated three locations in Saemankeun in pedological parameters - pH, organic matter, P2O5, SiO2, EC, T-N, Ex. Cation and CEC. Also, soil mesofauna groups, such as Diplura, Homoptera, Coleoptera, Dilptera, Lepidoptera, Hymenoptera, Collembola, Acari, Annelida, etc, have been examined. We analyzed the change of soil quality and the emergence of soil animals in newly formed Saemankeum reclaimed land in Korea.

Keywords:*fauna, soil characteristics, reclaimed region*

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O703F16

Entomology: General

O703

The role of urban wastewater farms as a common denominator for malaria and diarrhoeal disease transmission

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Worldwide, wastewater use in urban agriculture is increasing at a rapid pace due to rapid urbanization and its accompanying stress on limited freshwater resources. The economic and food security benefits associated with wastewater use in urban agriculture are enormous, but so also are the public health burden it exacts on the urban population. Wastewater use in agriculture has been implicated for the transmission of diarrhoeal diseases among farmers and their family members using wastewater and consumers of the wastewater farm products. Malaria mosquitoes also breed in wastewater farms, thus exposing urban populations living in close proximity to these farms to increased malaria risk. The problem of malaria is further exacerbated by the use of agro-chemicals on the wastewater farms that make malaria mosquitoes more resistant. So far, little is known of the role of wastewater farms for the combined risk of diarrhea and malaria. An understanding of this role would be important for developing integrated interventions. The main aim of this study is to assess the role of urban wastewater farms as a common denominator for the transmission of malaria and diarrhoeal disease in urban Ghana. The study is being conducted in Kumasi, the second largest city in Ghana, where more than 12000 hectares of urban vegetable farms are irrigated with wastewater. The outcome of the study will lead to the development of integrated interventions for mitigating malaria and diarrhoeal disease transmission associated with wastewater irrigation.

Keywords:*Diarrhoea, Malaria, Wastewater farms, Urban and Peri-Urban Ghana*

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PS1M001

Taxonomy

P1

Madagascar's millipede assassin bugs (Hemiptera: Reduviidae: Ectrichodiinae): taxonomy, phylogenetics, and sexual dimorphism

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[1]*University of California - Riverside, U.S.A.*, [2]*University of California - Riverside, U.S.A.*

Millipede assassin bugs in the subfamily Ectrichodiinae (Hemiptera: Reduviidae) are a diverse group (>600 species) of specialized millipede predators, with only 9 species documented from Madagascar, one of the world's premier biodiversity hotspots of critical conservation priority. The California Academy of Sciences' Terrestrial Arthropod Inventory of Madagascar Project has been established to address the need for taxonomic exploration of Malagasy arthropods and has provided a unique opportunity to examine >1,400 specimens of ectrichodiines and explore delimitation of new taxa based on male specimens. After examining 90 external morphological and genitalic characters, it is apparent that the Malagasy ectrichodiine fauna comprises more than 40 species, many of which represent new species. Ectrichodiinae are unusual among Reduviidae in that species frequently display strong sexual dimorphism, rendering association of males and females difficult. To associate females with males, an uncorrected pairwise distance analysis using ~430 bp of partial mitochondrial cytochrome c oxidase subunit I DNA sequences were analyzed, unambiguously matching some females to males. The first molecular dataset (COI, 28S D2-D3 rDNA; 60 taxa) of Ectrichodiinae was assembled to test generic concepts, as well as to assess the monophyly of Malagasy ectrichodiines. Results show that phylogenetics is an important tool to inform decision-making in taxonomy and that a small amount of sequence data can impact our ability to match dimorphic sexes in Reduviidae.

Keywords:*Ectrichodiinae, Madagascar, Taxonomy, Phylogenetics, Sexual Dimorphism*

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PS1M002

Taxonomy

P1

Phylogenetic reconstruction of the *Papilio memnon* complex suggests multiple origins of mimetic colour pattern and sexual dimorphism

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Mimicry refers to profitable/unprofitable organism benefiting from sharing conspicuous signals with unprofitable species. The evolutionary origin of mimetic characters is still a complicated mystery, especially when polymorphism and sexually dimorphism are involved. Papilio memnon, which the females exhibit at least 17 polymorphic forms, was considered as mimics of Troidini swallowtail butterflies and has been regarded as classical textbook case of Batesian mimicry. Having examined a long series of the museum material and observed field populations from different geographical areas, however, we found that most female forms of P. memnon do not seem to be involved in any mimicry ring. This may imply that female-limited polymorphism is not necessarily correlated with mimicry. To investigate whether the mimetic characters evolve from a single origin and whether the characters contributing to mimicry have correlated evolution, we reconstructed the Bayesian phylogeny of most of the species assigned to the species group and other species of the subgenus Melelaides, and a maximum likelihood model was used to trace the evolutionary history of 3 major character groups. The results show that: (1) P. memnon complex may be not a monophyletic because P. polymnestor is clustered within the clade; (2) no directional evolutionary trend from imperfect to accurate mimicry is observed; (3) the red basal patch on forewing underside occurs twice independently in P. memnon and P. thaiwanus; and (4) the mimicry associated with a specific pattern is area-dependent. Occurrence of a large number of non-mimetic female forms requires interpretation of other hypothesis, e.g. male mate-mate selection.

Keywords:*imperfect mimicry, Batesian mimicry, sexual dimorphism, polymorphism*

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PS1M003

Taxonomy

P1

The evolution of host utilization and shelter building behavior in the genus *Parapoynx* (Lepidoptera: Crambidae: Acentropinae)

Ling-Ying Tsai[1], Chia-Hsuan Wei[2], Shen-Horn Yen[3]

[1]National Sun Yat-Sen University, Kaohsiung, Taiwan, [2]National Sun Yat-Sen University, Kaohsiung, Taiwan, [3]National Sun Yat-Sen University, Kaohsiung, Taiwan

The genus Parapoynx is a medium-sized crambid group with a global distribution except for Antarctica. The caterpillars are truly aquatic, bearing branched tracheal gills and making portable cases using hostplant leaves. Our rearing experience and host acceptance tests reveal that caterpillars of different species-groups associate with aquatic plants of specific growing forms rather with closer phylogenetic affinities. This phenomenon is remarkable because most studies focusing on evolutionary trend between generalist and specialist herbivores specify on host association tied with plant's taxonomy, while this case suggests that ecological need is much more important in term of shaping host specificity. The present study aims to test two hypotheses: (1) If Agassiziella or Elophila is the sistergroup of Parapoynx? (2) If there is any evolutionary pattern of hostplant utilization within the genus? If yes, does it correspond to geographical or phylogenetic constraint? We applied Bayesian inference to reconstruct a phylogenetic tree of selected Acentropinae genera and Schoenobiinae species as an outgroup using COI (695 bp) and Ef1-α (666 bp). We also used adult morphological characters from nearly all the recognized species-groups to reconstruct a phylogeny of the genus using Maximum Parsimony. Our results reject the hypothesis either Agassiziella or Elophila is the sistergroup of Parapoynx. Most of the sampled Parapoynx are confined to certain plant growing types. However, species utilizing the same plant growing form are not distributed in the same geographical region or having closer phylogenetic relationships. Our results also suggest that hostplant form may have shaped the case-making behavior of Parapoynx.

Keywords:*aquatic macrophyta, plant growing form, polyphagy, aquatic caterpillar, shelter-building behavior*

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PS1M004

Taxonomy

P1

Phylogenetic analysis of the spider mite family Tetranychidae

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The spider mite family Tetranychidae comprises about 1,200 species, including the most injurious plant-feeding mites, and represents one of the most cosmopolitan and economically important groups of terrestrial arthropods. As for many other organisms, molecular information of spider mites is increasingly used to answer taxonomic and systematic questions. The cytochrome c oxidase subunit I (COI) gene of mitochondrial DNA and the internal transcribed spacer (ITS) of nuclear ribosomal RNA (rRNA) gene are being used for species identification and phylogenetic reconstruction within the family Tetranychidae. Although COI and ITS regions are effective for species identification between closely related species, these sequences have not consistently resolved genus-level phylogenetic relationships. On the other hand, rRNA gene sequences may be more useful for higher-level phylogeny. Therefore, we determined sequences of 18S and 28S regions, of spider mite species belonging to the 15 tetranychid genera and examined their phylogenetic relationships. Some genera, such as Aponychus and Panonychus, appeared to be monophyletic. The phylogenetic tree based on 18S and 28S sequences separates the Oligonychus species into two clades (I and II), with the Tetranychus species included in clade II. Thus, Oligonychus and Tetranychus were not separated using 18S and 28S rRNA genes.

Keywords:*Tetranychidae, phylogenetic analysis, DNA, aedeagus*

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PS1M005

Taxonomy

P1

A pteromalid (Hymenoptera: Chalcidoidea) parasitizing larvae of *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) and the first finding of the facial pit in Chalcidoidea

Kazunori Matsuo[1], Junichiro Abe[2], Kanako Atomura[3], Junichi Yukawa[4]

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An endoparasitoid emerged from pupae of an aphidophagous cecidomyiid Aphidoletes aphidimyza in Japan. Examination of morphological features revealed that the endoparasitoid was identical to Gastrancistrus fuscicornis (Pteromalidae) that has been known from European countries. The host insect of G. fuscicornis was thought to be an unidentified aphid because three females in the Hartig's collection have been mounted with the skins of parasitized aphids on some rose leaves. However, no species of the genus have been reared from aphid mummies up to the present. This information supports the current result that G. fuscicornis is not an aphid parasitoid but larval-pupal parasitoid of A. aphidimyza. During the course of morphological observation, we found a pit on the head of male and female of G. fuscicornis. Examination of internal cuticular structure revealed that a bell shaped internal cuticular apodeme was connected to the pit but not to the tentorium. According to the definition of the non-tentrial pits, the pit found in G. fuscicornis corresponds to the facial pit. This is the first finding of the facial pit in Chalcidoidea.

Keywords:*Gastrancistrus fuscicornis, predacious gall midge, Japan.*

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PS1M006

Taxonomy

P1

Population genetics of common Palearctic solitary bee *Anthophora plumipes* (Hymenoptera: Anthophoridae) in whole species areal and result of its recent introduction in the USA

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Anthophora plumipes (syn. A. pilipes) is a common polylectic early spring solitary bee, that nests in aggregations. The species is often synathropic and thus well known. It is spread across the whole Europe, North Africa, Middle East and East Asia (www.discoverlife.org). As a promising pollinator, A. plumipes was introduced to the USA in Maryland from Japan (Sugata-Cho, Matsue) in 1989. Originally, 109 diapausing adults were shipped from Japan from which 56 adults were used for the introduction. Bees were kept outdoors for five years and population approximately tripled each year (Batra, 1994). We have obtained material of A. plumipes from the eastern coast of the USA, where it is nowadays rather abundant, to perform the population-genetic analysis of this species, because with its known history it represents very interesting evolutionary model. We further collected population samples from the most parts of its areal range, which enabled us to perform complete population-genetic analyses of this species in the whole areal. We used several novel microsatellite loci developed for this purpose (Cerna & Straka, 2012) and also performed analysis on mitochondrial gene COI. Our results show interesting features concerning both populations genetic of this species as well as the estimation of success of presented introduction of A. plumipes to the USA. Literature cited: Batra S. (1994): Proc. Entomol. Soc. Wash. 96: 98-119. Cerna K. & Straka J. (2012): Eur. J. Entomol. 109:155-160.

Keywords:*Anthophora plumipes, population genetics, species introduction, microsatellites, COI*

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PS1M007

Taxonomy

P1

Multiple nuclear and mitochondrial DNA analyses support a cryptic species complex of the global invasive pest, *Bemisia tabaci* (Gennadius) (Insecta: Hemiptera: Aleyrodidae)

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Cryptic species is an important issue to understand the biodiversity and evolutionary biology. Detection of the species status influences the conservation decision and invasive pest management. DNA sequences are powerful tools to discriminate morphologically indistinguishable species. Multiple nuclear and mitochondrial genes and multiple outgroups were used to detect the species status of the global invasive pest, Bemisia tabaci. Phylogenetic analyses revealed B. tabaci clustered with several Bemisia species and not a monophyletic group. The topologies indicated the paraphyletic origin and three phylogroups of B. tabaci. Intraspecies genetic divergent of B. tabaci was higher than interspecies of Bemisia species (Lipaleyrodes group). High genetic divergence between genetic groups of B. tabaci suggested at least each genetic group was a genetic species. Bayesian species delimitation detected the reproductive isolation between genetic groups of B. tabaci and supported each genetic group was a biological species. Isolation with migration model was used to estimate the gene flow and no significant gene flow was observed between closed related genetic groups of B. tabaci. Sympatric distribution of two different genetic groups of B. tabaci were observed in a poinsettia greenhouse, microsatellite analyses showed strong reproductive isolation between them and provided direct evidence to support a cryptic species complex. Combination of multiple loci was first used to infer the species status of B. tabaci and suggested a cryptic species complex based on genetic species concept and biological species concept. Cryptic species status is important to understand the cryptic biological invasion and help to formulate the invasive pest control.

Keywords:*multiple nuclear loci, DNA barcoding, genetic species concept, biological species concept*

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PS1M008

Taxonomy

P1

Diverse neuropterans in the Mesozoic Ecosystems of Northeastern China

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Mesozoic Neuroptera shows most species richness and morphological diversity in the Northeastern China, suggested it should have been a golden time for neuropterans in Mesozoic Era. Up to date, 18 families, 42 genera and 56 species have been described in Mesozoic China, and mainly found in the Jiulongshan Formation and Yixian Formation. Based on morphological data, incorporating most fossil and extant families, the familial phylogenetic relationships of Neuroptera were re-constructed. Four extinct families, Permithonidae、Archeosmylidae、Mesithonidae and Nevrorthidae constitute the stem-groups of Neuroptera, representing the earliest division of Neuroptera. The suborder Myrmeleontiformia is confirmed, and Saucrosmylidae, Kalligrammatidae, Panfiloviidae, Aetheogrammatidae and Grammolingidae are assigned to the suborder. The superfamily Chrysopoidea is corroborated, comprising of Limaiidae、Tachinyphidae、Allopteridae、Mesochrysopidae and Chrysopidae. According to the fossil records of Neuroptera, it is inferred the familial division of Neuroptera is likely at the Middle Jurassic. The Mesozoic fossil lacewings exhibit some specialized co-evolution relationships with the contemporaneous plants and environments that have been lost in the Modern insects, etc. the oldest pinnate leaf-mimicking insects, Bellinympha filicifolia and B. dancei reveal a specialized co-evolution of insects and gymnosperms. Other extinct groups: Kalligrammatidae, Grammolingidae and Saucrosmylinae show some special traits that are different from the modern neuropterans, e.g. extremely large body size, peculiar wing marks and special behavioral adaptation. These enigmatic insects implied the diverse living strategies of Neuroptera before adaptation of angiosperms. The research of Mesozoic neuropterans would promoted our knowledge to understand the early evolutionary history of Neuroptera.

Keywords:*Neuroptera, Mesozoic, fossil, diversity*

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PS1M009

Taxonomy

P1

A developmental approach to the evolution of female wing regression in bagworm moths (Lepidoptera, Psychidae)

Shuhei Niitsu
Tokyo Metropolitan University, Japan

Bagworm moths (Lepidoptera: Psychidae) include approximately 1000 species in the world, all of which complete the larval development within a self-enclosing bag. They show one of the most extreme cases of sexual dimorphism in wing development; females of some species lost their wings completely in relation to their case-dwelling life style, but females of the other species did not. The loss of wings is achieved by: 1) loss of the potential of cell proliferation during wing disc development (subfamily Oiketicinae); 2) wing bud regression caused by apoptosis in the larval and the pupal stages (subfamilies Taleporinae, Psychinae, and Epichnopteriginae). In this study, to examine the evolutionary pattern of such a female wing-regression, we reconstructed a molecular phylogeny of over 20 species of Asian Psychidae based on the sequences of mitochondrial COI and nuclear 28S rDNA. The results suggested that the groups of wingless females appeared from the group of winged females and that the wing loss of the female adult is achieved through different developmental processes that occurred multiply.

Keywords:*Bagworm moth, Psychidae, wing regression, sexual dimorphism, evolution*

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PS1M010

Taxonomy

P1

Formation of abdominal appendage-like swellings in the *Carabus insulicola* embryo (Coleoptera, Carabidae): The possible relationship to the aquatic origin of Adepahga

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The successive changes of developing embryos of Carabus insulicola were described from observations based on light and scanning microscopy. Despite of the absence of larval abdominal legs, two pairs of appendage-like swellings, the medial and lateral ones, appear on both sides of each of the first eight abdominal segments of the middle stage embryo. The distal parts of medial swellings of the first abdominal segment later develop into large cup-shaped pleuropodia. As development proceeds, however, the medial ones of the second to eighth abdominal segments become flattened. In the newly hatched larvae, the flattened parts almost correspond to the abdominal hypopleura. The lateral ones also soon become flattened and disappear. Regarding the pleuropodia as the structures being homologous with thoracic legs, the medial swellings of the second to eighth abdominal segments are also homologous with the proximal parts of the thoracic legs, probably coxal parts. The lateral swellings originate in more proximal parts, or subcoxal parts, of the medial swellings. In the newly hatched larvae, the subcoxal parts become the abdominal epipleura; thus, the lateral swellings can be regarded as temporal subcoxal projections. The nature of the lateral swellings is obscure. However, considering the subcoxal origin of the larval tracheal gills in the Gyrinidae embryos suggested by our studies, we can speculate that the lateral swellings are the vestiges of subcoxal tracheal gills, and the ancestral larvae of the Carabidae might have had tracheal gills. This interpretation may provide information favorable to the assumption that the ancestral adephagan was aquatic.

Keywords:*Carabus, Carabidae, Adephaga, embryo, larva*

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PS1M011

Taxonomy

P1

South American "jewel acrolepiids": A molecular phylogeny based on 8 genes reveals they represent a novel lineage of Argyresthiidae (Lepidoptera: Yponomeutoidea)

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Neotropical yponomeutoids are one of the most poorly studied lepidopteran faunas, with many groups still tentatively defined. One example is the genus Dasycarea Zeller, 1877, based on a single female specimen of the type species D. viridisquamata from which the abdomen is missing. Due to the lack of information about the genitalia, different authors have assigned very different taxonomic placement to Dasycarea: Gelechiidae by Zeller (1877), Roeslerstammiidae by Heppner (1984) and Acrolepiidae by Becker (1999). Reexamination of the type species of Dasycarea revealed that it is closely related to at least eight species of South American yponomeutoids which were misidentified as acrolepiids. An updated inventory of the Neotropical yponomeutoids has found over thirty new species belonging to Dasycarea. To define their systematic position, a molecular phylogenetic analysis was performed based on eight nuclear genes, totaling 8,096bp. The resulting tree showed, in contrast to all previous proposals, that Dasycarea is the sister group to Argyresthia, currently placed in its own family, Argyresthiidae. This association is tested by morphology and receives further support.

Keywords:*Lepidoptera, Molecular Phylogeny, Yponomeutoidea, Acrolepiinae, Argyresthiidae, Neotropical Region*

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PS1M012

Taxonomy

P1

Braconidae and Ichneumonidae (Hymenoptera) associated with corn pests *Spodoptera frugiperda* (Lep., Noctuidae) and *Dichomeris famulata* (Lep., Gelechiidae) with descriptions of new species.

Angelica Penteado Dias[1], Ivy Frizo Melo[2], Rafael Braga Silva[3], Ivan Cruz[4]

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The role and importance of biodiversity in agro-ecosystems is widely recognized and it can be used to improve pest management, providing a means of determining the abundance and dynamics of individual species. A major problem in areas of agriculture is the lack of basic research on distribution, abundance and taxonomy of insect pests and their enemies. Spodoptera frugiperda and Dichomeris famulata are important pests on corn crops, widely distributed, presenting, in Brazil a complex of parasitoids with occurrence and distribution poorly studied. The aim of the present work is to update the bionomic and taxonomic knowledge of the S. frugiperda and D. famulata parasitoids from Brazil. Samples of larvae were held during 2010-2011 at the Minas Gerais State, Brazil along the cropping season. The larvae were placed individually in glass tubes, fed with artificial diet, until the emergence of parasitoids identified by two of the authors (AMPD and IFM). From S. frugiperda emerged: Campoletis flavicincta, Ophion flavidus, Eiphosoma laphygmae, Colpotrochia sp. nov. (Ichneumonidae) and Chelonus (Chelonus) insularis, Dolichozele koebelei, Exasticolus sp nov. (Braconidae). The species of Dolichozele and Colpotrochia are recorded for the first time parasitizing S. frugiperda. From D. famulata emerged the Braconidae : Cotesia sp., Glyptapanteles sp., Phanerotoma sp.(fuscovaria group) and Chelonus (Microchelonus) sp. nov. (first occurrence on Dichomeris famulata). Those parasitoids are koinobiont endoparasitoid solitary or gregarious and emerged from larvae or pupae of the hosts. The material is deposited in the Brazilian Collection (DCBU) Sao Carlos, SP. The species are illustrated with digital and SEM images.

Keywords:*parasitoids, biological control, corn pests, biodiversity, Brazil*

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PS1M013

Taxonomy

P1

New contributions to knowledge of pimplinae wasps (Hymenoptera, Ichneumonidae) from Brazil

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This study aimed to contribute to the knowledge of Neotropical fauna of Pimplinae through the characterization and quantification of their genera and species in Southeast of Brazil. Using mainly Malaise traps in 11 localities in the State of Sao Paulo, from 2006 to 2008, we collected 716 specimens of Pimplinae, distributed in 58 taxa , including 15 genera and 33 species (25 possible new species) and new records (14) for the Brazilian fauna. In the tribe Ephialtini were identified specimens of the groups of genera Ephialtes (Scambus, Calliephialtes), Camptotypus (Clydonium, Zonopimpla), Tromatobia (Tromatobia, Clistopyga and Zaglyptus) and Polysphincta (Polysphincta, Acrotaphus, Hymenoepimecis, Eruga and Zatypota) . In the tribe Pimplini, we obtained specimens of the groups of genera Theronia (Neotheronia) and Pimpla (Pimpla). Neotheronia and Hymenoepimecis were the genera with the largest number of species identified, 16 and 9 respectively. Pimpla, Neotheronia and Acrotaphus were the most frequently sampled making up more than 80% of all material, while Calliephialtes, Scambus and Eruga made only 0.56%. We obtained 140 specimens of Pimpla croceiventris, about 19.55% of total and Neotheronia lineata accounted for 17.46% of total of the individuals collected. Regarding the analysis of seasonality, considering the dry season period from April to September and the rainy season from October to March, we have found that the frequencies of occurrence of the species were higher in samples collected during the rainy season. The material is deposited in Brazilian Collection (DCBU) Sao Carlos, SP. Maps of distribution and characterization of the species are presented.

Keywords:*Hymenoptera, Ichneumonidae, Pimplinae, Brazil, biodiversity*

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Taxonomy

P1

Myrtlemiris, a new genus and new species of Australian plant bugs (Insecta: Heteroptera: Miridae): Systematics, phylogeny and host associations

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A new Australian genus of Orthotylinae, Myrtlemiris Cheng, Mututantri & Cassis gen.n., is described, with nine included species described as new to science (M. agnew sp.n., M. astartephila sp.n., M. meanarra sp.n., M. newmanensis sp.n., M. rubrocuneatus sp.n., M. russulatus sp.n., M. silveirae sp.n., M. tesselatus sp.n., M. yalgoo sp.n.). A phylogenetic analysis based on 39 morphological characters is presented for all Myrtlemiris species and 6 outgroup taxa. This analysis establishes Myrtlemiris as monophyletic, defined by a broad apophysis on the left paramere. A key to species is provided and diagnostic characters are illustrated. Myrtlemiris is endemic to southwest Western Australia. Host plants for Myrtlemiris species are near restricted to the myrtaceous tribe Chamelaucieae.

Keywords:*Orthotylini, host plants, Chamelaucieae, phylogenetics, Western Australia*

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PS1M015

Taxonomy

P1

Morphological antennal sensilla in some importance forensic flies In Saudi Arabia

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We investigated the sensilla of the antenna of the some adult flies, Parasarcophaga sp , Musca domestica , Chrysomya megacephala, belonging to Calliphoridae, Sarcophagidae and Muscidae using Scanning Electron Microscopy. Many types of sensilla were observed; no marked differences in morphological structure and distributional pattern of any sensilla among these species with the exception of more numerous sensory pits were found. The suggested function of each antennal sensillum would be based on the comparison with other studies of similar sensilla.

Keywords:*Calliphoridae, Sarcophagidae, Muscidae, Parasarcophaga sp, Musca domestica, Chrysomya megacephala*

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PS1M016

Taxonomy

P1

A revision of the genus *Morophagoides* Petersen (Lepidoptera, Tineidae) in Japan

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The genus Morophagoides Petersen, 1957 consists of 9 species globally, distributed in the Palaearctic, Oriental (Taiwan), Nearctic and Neotropical regions, and some species are well known as pests of the Shiitake mushroom Lentinus edodes (Robinson, 1986). Larvae of this genus injure the bed log and fruit body of the mushroom, and produce a remarkable degree of damage. In order to develop applied methods of controlling the pests using a pheromone or natural enemy, it is necessary to identify species correctly. In Japan, Morophagoides moriutii Robinson, 1986 is known as an important Shiitake mushroom pest (Moriuti, 1976. Inoue, 1988). The larvae feed on the mushroom, and its bed log (Moriutii, 1976). However, the taxonomy of the genus is complicated and controversial. In this study, we found that the genus consists of 9 species, including M. moriutii, M. ussuriensis, and an additional 7 new or unrecorded species in Japan. The 9 species are grouped into 5 species groups based on the male and female genitalia. Emergence time and distribution of the species are compared from the label data of the specimens.

Keywords:*Lepidoptera, Tineidae, Morophagoides, taxonomy, Shiitake mushroom*

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PS1M017

Taxonomy

P1

Comparative study of hind-wing venation of Cerambycinae (Coleoptera: Cerambycidae) from China

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A comparative study on hind-wing venation was performed based on 115 species, 53 genera, 18 tribes of the subfamily Cerambycinae. This study finds not only significant differences in venation features of the Cerambycinae at tribe-level and genus-level, but also the evolutionary trends of Cerambycidae. The chosen venation characters could be used in identification of Cerambycinae. The key of Cerambycinae based on characters of hind-wing venation was presented. Cerambycinae could be divided into three types by variation of MP3+4 and CuA1+2 (① Trace of CuA1+2 is clear, fuses with MP4. These two fusing branches reach the posterior margin. ② no trace of CuA1+2, MP3+4 forks into MP3 and MP4. MP4 reaches the posterior margin. ③ no trace of CuA1+2, no forks of MP3+4. Only MP3+4 reach the posterior margin), which showed the most important information of hind-wing in Cerambycinae, and the transition types in wing morphology appeared to reflect evolutionary trends. But venation could be only used for identification of a few species within transition types. We consider the venation nomenclature proposed by Kukalova-Peck and Lawrence (2004) for r4, CuAF, and relation of AA3+4 and cubitus shouldn't used in Cerambycinae. However, the part of cross-vein by Salaas (1936) and Suzuki (1969) is a nice way to solve these questions. So, we consider the best method to make these venation clear is combine the main veins of Kukalova-Peck and Lawrence (2004) and the cross-vein of Salaas (1936) and Suzuki (1969)

Keywords:*hind-wing venation, Cerambycidae, Cerambycinae, China*

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PS1M018

Taxonomy

P1

Occurrence of an undescribed *Metapocyrtus* species attacking *Hedera helix* in Japan (Coleoptera: Curculionidae: Entiminae)

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National Institute for Agro-Environmental Sciences, Japan

Globalization facilitates the spread of pests and alien species throughout the world. In 2010, an undetermined weevil (Coleoptera: Curculionidae) caused serious damage to the cultivated ivy, Hedera helix (Araliaceae), in a farm of ornamental plants in Kuwana County, Mie Prefecture, Central Honshu, Japan. Adult weevils attacked leaves and stems of H. helix, whereas its larvae attacked the roots in the soil. Judging from its morphological features, this pest was considered as a species of the subgenus Trachycyrtus, genus Metapocyrtus (Entiminae: Pachyrhynchini), which comprise about 220 species from Southeast Asia, but it was unable to be identified at species level based only on literature data. In order to identify this pest weevil, I examined major reference collections of Metapocyrtus including many type specimens at the Museum fur Tierkunde, Dresden and the Natural History Museum, London. After careful examination, I concluded that the species in question is different morphologically from all known species and thus new to science. This species, which occurred suddenly and locally in an artificial environment, is considered not to be native to Japan but an invasive species from a Southeast Asian country, most probably from the Philippines that contains the great majority of Metapocyrtus species. However, the invasion route of this species to Japan is still unclear.

Keywords:*Taxonomy, Pachyrhynchini, Trachycyrtus, Agriculture, pest, alien species, Ivy*

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PS1M019

Taxonomy

P1

Bagworms (Lepidoptera: Psychidae) of the European part of Russia

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All-Russian Center for Plant Quarantine, Russia

Bagworms (Psychidae) is a moderate family of Lepidoptera, which numbers more than 1300 species in the world. The bagworms fauna of many countries of the world is studied quite well, while the bagworms fauna of the European part of Russia was not researched. In this work bagworms of the European part of Russia were investigated. My research is based on own materials, museum collections and literature records. In the course of our investigation we establish that four species Diplodoma taurica, Praesolenobia clathrella, Dahlica wockei, Postsolenobia banatica must be excluded from the checklist of bagworms of the European part of Russia, because their inclusion was a result of a misidentification or incorrect reference. While, two species were recorded for the first time. New generalized checklist of bagworms of the European part of Russia containing 65 species from 32 genera was made . The most numerous subfamilies are Narycinae and Oiketicinae, each of them includes 18 species. The least numerous subfamily are Typhoniinae that includes 2 species. Also subfamilies Taleporiinae (5 species), Psychinae (9 species), Epichnopteriginae (13 species) are represented. Also were found some new records for different regions of the European part of Russia, for example Psychidea alba was found for the first time in the Caucasus, Apterona helicoidella and Rebelia nocturnella was found for the first time in the center of the European part of Russia. Also in the course of my study a special key for identifying males and females was created.

Keywords:*Psychidae, European part of Russia, checklist*

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PS1M020

Taxonomy

P1

"What is *Tipula (Nippotipula) coquilletti*?": Determined by the holotype examination (Diptera: Tipulidae).

Takashi Torii
Keio University, Japan

Tipula (Nippotipula) is the crane fly subgenus containing mainly large to extra large species, which are distributed in the eastern Palaearctic, Oriental, and eastern Nearctic Regions. Only one species, i.e., T. (N.) coquilletti was recorded from Japan. No information was given on male terminalia in the original description of T. (N.) coquilletti, and no one examined the holotype except the original author. In the present study, a species named T. (N.) sp. 1 was recorded from several localities of Honshu, Japan. The species was closely related to T. (N.) coquilletti in the sensu of several authors (of which no one examined the holotype), but distinguished from the latter only by the details of structures of male terminalia. However, because of the lack of information on male terminalia for T. (N.) coquilletti, it was impossible to determine which is the true T. (N.) coquilletti: tentatively identified T. (N.) coquilletti by the above authors, or T. (N.) sp. 1. The present author had the loan of the holotype of T. (N.) coquilletti from the National Museum of Natural History, USA, and determined that tentatively identified T. (N.) coquilletti is the true T. (N.) coquilletti. The detailed redescription of the species is given in the present study, especially on male terminalia. In addition to this, T. (N.) sp. 1, which is new to Japan or to science, is described as compared with resembling species, T. (N.) sinica, which is recorded from Sichuan and Zhejiang, China.

Keywords:*Tipula (Nippotipula) coquilletti, holotype examination, male terminalia, Tipula (Nippotipula) sp. 1, new to Japan or to science*

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PS1M021

Taxonomy

P1

Divergence time estimation of fern-feeding sawflies

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Most of vascular plants are used to some extent as a food source by phytophagous insects. However, these groups are not used equally, and the Filicales is rarely attacked by members of Insecta. The ratio of fern-feeding insects to fern species in number is lower than that of phytophagous insects to angiosperm species. The extraordinary diversification of angiosperms throughout the Cretaceous and Tertiary, culminating in an estimated 250,000-300,000 living species, is considered. The extraordinary diversification of living insects has been attributed by some workers to the diversity of angiospermous plants. This notion is supported by exhaustive examples of complex ecological coupling between the two groups, especially with regard to insect herbivory and pollination. While, ferns, with more than 10,000 living species, are the second largest group of vascular plants. Most of ferns are considered to be diversified in the Cretaceous. Does fern diversification promote fern-feeder insect diversification? Hymenopteran, especially symphytan, fern-feeders provide a good model system for evolutionary question because the proportion of fern feeders in phytophagous Hymenoptera is higher than in other insect orders. We reconstructed molecular phylogenetic relationships among 25 genera of sawflies, including novel several fern-feeder species of 6 genera, using Bayesian method. We used approximately 2kb of DNA sequence data sets. Most DNA sequence data were already available in GenBank. The fern-feeder was shown into two lineages and the range of divergence time of symphytan fern-feeder was 93-76 Mya calculated by the molecular clock analysis.

Keywords:*Symphyta, fern-feeding, Bayesian phylogeny, molecular clock,*

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PS1M022

Taxonomy

P1

Solitary and social halictine bees collected by the Kyushu University expeditions to Central Asia

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We conducted five surveys to accumulate faunal information on wild bees to assist planting campaigns in Central Asia (Kazakhstan, Kyrgyzstan) and neighboring area (northwestern China) from 2000 to 2004 under the project title "Field Studies of Wild Bee Fauna and Pollination Biology for Combating Desertification and Planting Campaign in Asian Arid Areas" (Tadauchi, 2005). A total of 21,494 bee specimens were collected from Central Asia by this project. The taxonomic result based on this project have been published in following taxa: Colletes (Kuhlmann, 2009), Andrena (Tadauchi et al., 2005; Tadauchi, 2006, 2008; Shebl & Tadauchi, 2009, 2011), Nomada (Mitai & Tadauchi, 2008), and Bombus (Williams, 2011). However, the bee family Halictidae which mostly occupied in the collection (collected 16,613 specimens) is not well studied yet. Recently, one of author's Murao visited to some of European and Russian Museums to study and identify the halictine bees specimens (excluding cleptoparasitic genus Sphecodes) collected by this project. In our presentation, we report a taxonomic result about solitary and social halictine bees collected by the Kyushu University expeditions to Central Asia.

Keywords:*Taxonomy, Hymenoptera, Halictidae, Central Asia*

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PS1M023

Taxonomy

P1

Discrimination methods of a migratory pest, *Spodoptera exempta* occurred in Japan for the first time in 2010 from other similar Japanese pests of the genus *Spodoptera* Guenee (Lepidoptera: Noctuidae)

Daisuke Watabiki[1], Shin-ichi Yoshimatsu[2], Hiraku Yoshitake[3], Yuki Baba[4], Takumi Uesato[5], Masayuki Shimatani[6], Hiroshi Ibusuki[7], Tatsuya Yuda[8]

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The genus Spodoptera Guenee mainly distributed from tropical to subtropical regions throughout the world includes many pests of food crops, vegetables, forage crops and grasses. S. exempta has been known from Africa, South East Asia, parts of Australia to some Pacific islands as a pest of crops or pasture, however, no damage by this pest had been reported from Japan before 2010. From Japan, only five adult specimens had been collected before 2010, and probably they flew from tropical region of Asia to Japan because S. exempta is a well known migratory pest in Africa. Severe damage of forage crops and grasses by this species was recognized for the first time from Japan at Nansei Isls. in 2010. Therefore, survey using synthetic sex pheromone for S. exempta was conducted by the Okinawa Prefectural Plant Protection Center and the Kagoshima Prefectural Institute for Agricultural Development. As a result, several species of the genus Spodoptera were collected involving S. exempta by the pheromone trap for S. exempta. For convenience, we made a key to distinguish eight Japanese species of Spodoptera mainly using male genitalia. Furthermore, we could distinguish six Japanese pests of Spodoptera each other using the method of the standard DNA barcoding.

Keywords:*Spodoptera exempta, Japan, male genitalia, DNA barcoding*

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PS1M024

Taxonomy

P1

Genetic structure of *Aeolesthes oenochrous* (Coleoptera: Cerambycidae) in Taiwan

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Wushe blood-spotted longhorn beetle, Aeolesthes oenochrous (Fairmaire), feeding on domestic cherry tree (Prumus campamulata), is one of the endangered species in Taiwan. In the past decade, the beetle's habitat and population size have increased due to the plantation of cherry trees for tourism. Therefore, in spite of lacking information on the genetic structure and diversity of this species, a consideration of removing it from the conservation list has been proposed. The study herein presents analyses based on mitochondrial COI and nuclear ITS2 genes to investigate the population structure and ongoing gene flow of this beetle. Seven populations with 83 individuals were collected, and 19 haplotypes in COI showed 0.885 in haplotype diversity and 0.004 in nucleotide diversity. AMOVA showed significant COI divergence between populations (average Fst=0.4). In ITS2 sequences, a microsatellite of (CTA)n repeat was found ranging from 5 to 9. Phylogeny and network analysis showed that A. oenochrous populations can be separated into western and widespread clades, indicating two events of colonization or single colonization by the following differentiation has occurred. Moreover, the highest genetic diversity found in both COI and ITS2 sequences indicated that Taoyuan might be one of the possible ancestral populations. According to the population demography, individuals of two lineages occurring in the same localities might due to population expansion. However, a gene flow between populations might be also caused by human activities since dot plot distributions of genetic pairwised distances to their relevant geographical distances showed no positive relationship either in western or widespread clades.

Keywords:*conservation insect, COI, genetic variation, ITS2, gene flow*

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PS1M025

Taxonomy

P1

The phylogeny and host specificity of gall midges (Diptera: Cecidomyiidae) inducing similar spindle-shaped leaf galls on ten *Quercus* L. (Fagaceae) species in Taiwan

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The interaction between gall-inducing insects and their host plants is a complex part of the evolutionary scenario. In Taiwan, a group of allied gall midges induce similar spindle-shaped galls on leaves of 10 species of subgenus Cyclobalanopsis of genus Quercus (Fagaceae). Our preliminary observations found that a gall midge species may induce similar galls on different host plants; alternatively, different gall midge species are also able to induce similar galls. Thus, it would be desirable to make further investigations and to construct a phylogeny to help clarify the relationships among host plants, gall midges and gall morphology. In this study, molecular sequences of both mitochondrial gene COI and nuclear gene ITS2 from midge larva were used to build molecular phylogenetic trees. Morphological characters of galls were analyzed by ANOSIM similarity analysis for grouping and compared with molecular phylogeny. The result shows that gall midges on C. championii, C. gilva, C. hypophaea, C. morii and C. pachyloma are monophyletic groups and suggests each representing a distinct species. However, gall midges from C. globosa, C. longinux, C. stenophylloides are paraphyletic groups and mixed with samples from C. glauca which suggests these taxa may be recently diverged. The result of the similarity analysis of gall morphological characters was, for most part, consistent with the molecular phylogeny. Although the identification of gall midge species is not fully clarified, the results suggest that midges inducing spindle-shaped galls on Cyclobalanopsis reveal a trend of host specificity, and closely related clades induce similar galls.

Keywords:*gall midge, Cyclobalanopsis, molecular phylogeny, host specificity, gall morphology*

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PS1M026

Taxonomy

P1

Studies on geometridae larvae (Lepidopera) and its parasitoids in southeastern Brazil

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Larvae of Geometridae (Lepidoptera) were obtained in two areas with native vegetation recovery in Southeastern Brazil, Sao Paulo State. The samples were collected by entomological umbrella, for a minimum of one year. Larvae were kept in the laboratory on their host plants until the emergence of moths or parasitoids. Among the common Geometridae were some species of Macaria, Oxydia, Glena, Hymenomima, Ischnopteris (Ennominae) and Eois (Larentiinae). The Ennominae, in number of species, represents about half of the Geometridae and the results obtained here show the almost complete predominance of species of this subfamily; the Larentiinae and Sterrhinae (Semaeopus and Cyclophora) were not so common. The most common parasitoid species were Euplectrus (Eulophidae), Glyptapanteles, Diolcogaster (Braconidae) and Diradops, Charops (Ichneumonidae). Some of the parasitoids were for the first time found on larvae of Geometridae and included some new species. The studied material was deposited in the collection of the Department of Ecology and Evolutionary Biology, Federal University of Sao Carlos, Sao Carlos, Sao Paulo State, Brazil. Bionomic data on the species are presented. Seasonal variations were observed in the abundance of larvae of Geometridae, as well different relations of specificity of the larvae and their host plants. The greater occurrence of larvae was recorded in March, April and May, corresponding to the final period and immediately after rainfall. The environments of native vegetation recovery were important for the establishment of populations of species of Geometridae and can act as links between fragments of natural vegetation remaining in this region.

Keywords:*GEOMETRIDAE, LARVAE, PARASITOIDS, NEOTROPICAL, BRAZIL*

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PS1M027

Taxonomy

P1

Phylogeography of four stag beetle groups (Coleoptera, Lucanidae) in the Ryukyu Archipelago, Japan

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Four different groups belonging to the family Lucanidae distributed in the Ryukyu Archipelago and adjacent regions were examined to evaluate each phylogeography, based on the mitochondrial 16S ribosomal RNA gene sequence. The resultant tree of the subgenus Psalidoremus of the genus Prosopocoilus contains two major lineages: one consists of the members collected from mainland Japan and the Osumi Islands, and another from the Ryukyu Archipelago and Taiwan. The latter is further divided into the following two sublineages: the Tokara Islands and the Central Ryukyus, and the Southern Ryukyus and Taiwan. The nucleotide substitutions between them as well as those between two major lineages show a relatively high percentage. The resultant trees both Neolucanus maximus species group and Dorcus amamianus show similar patterns that of the subgenus Psalidoremus living in the Ryukyu Archipelago, with two major lineages, the central and southern Ryukyus, respectively. These common patterns in phylogeography of different three lucanid groups suggest involvement of these lucanid beetles in a series of vicariances in the Ryukyu archipelago and Taiwan regions during the late Pliocene and the early Pleistocene. On the other hand, the resultant tree of Dorcus titanus contains two major lineages: one consists of the Tokara Islands and Amami Island in the central Ryukyus, and another of the remaining regions of the Ryukyu Archipelago, Taiwan and mainland Japan. The nucleotide substitutions between two major lineages show a relatively low percentage. These results indicate that recent migration of Dorcus titanus complicated its current distributional patterns.

Keywords:*Biogeography, Ryukyu Archipelago, Lucanidae, vicariance*

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Taxonomy

P1

Evaluation of the secondary structure of 16S rRNA for molecular phylogeny of Schizophora clade (Diptera: Brachycera)

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The 16S is the largest ribosomal RNA subunit of the mitochondrial DNA and is extensively employed in evolutionary studies of insects. Nevertheless, its structural information has been considered only recently. In this work, the sequence and secondary structure of the 3'-half of the 16S (535 bp) were evaluated as molecular marker for phylogenetic studies of twenty-nine dipteran species of Schizophora clade. The Schizophora comprises one of the greatest insect radiations, but molecular phylogenetics usually concerns its ingroup relationships, highlighting the need for further studies on different hierarchical levels. In this context, secondary structure prediction was performed in silico and based on homology of conserved structural motifs. Phylogenetic signal analyses showed that 3'-half of the16S have appropriate information for phylogeny of hierarchical levels analyzed, increasing when double-stranded sites are considered. For Calyptrate, Bayesian inferences using sequence and structure, supported the monophyly of the superfamily Oestroidea (PP 0.91) and its placement as a sister group of the superfamily Muscoidea. The Rhiniidae have been raised to family status recently and the species used in this work presented two placements in the trees recovered: (Rhiniidae, (Oestridae+Tachiniidae)) and (Sarcophagidae+Rhiniidae). The monophyly of Acalyptratae was not supported and interfamilial relationships recovered the Lauxaniidae as a sister group of (Sepsidae+Drosophilidae) (PP 0.44) and (Sphaeroceridae, (Ropalomeridae, (Micropezidae + Aulacigastridae))) (PP 0.91). Our results indicates that the use of sequence and structure of 16S increase the phylogenetic signal for Schizophora old divergence groups, but presents limitations in some taxonomic levels, probably related to the rapid diversification of schizophoran lineage.

Keywords:*rRNA 16S, mtDNA, Diptera, Schizophora, secondary structure*

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PS1M029

Taxonomy

P1

Taxonomic study on *Cryptocercus* Scudder

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The present research indicated there were some incorrect identification in previous study, for instance, the species represented by 16S rRNA (DQ007646), COII (DQ007643) and the species represented by 16S rRNA (DQ007647), COII (DQ007644) should not be conspecific, but they were considered as the same species previously, while the species represented by 12S rRNA (AY631411), 16S rRNA (AY631410) and the species represented by 12S rRNA (AJ519679 in EMBL), 16S rRNA (AJ519678 in EMBL) should be conspecific. We used a single gene and combined two or three genes to build phylogenetic trees (methods by Parsimony, Maximum Likelihood and Bayesian). Two new species of the genus Cryptocercus Scudder 1862 were described based on morphological characters and DNA sequence of the three mitochondrial genes (partial sequence): 12S rRNA, 16S rRNA and cytochrome oxidase subunit II (COII). One species was collected from Shennongjia in Hubei Province and mountain Taibai in Shaanxi Province and another from mountain Tianhua in Shaanxi Province. All these places were newly discovered distributions to Cryptocercus.

Keywords:*New species, New distributions, Cryptocercus, China*

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PS1M030

Taxonomy

P1

Analyses of the mating behavior of the Japanese *Auplopus carbonarius* species-complex (Hymenoptera, Pompilidae)

Hiroaki Kurushima[1], Akira Shimizu[2]

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Members of Auplopus are mud-daubers, which plaster mud in nest construction. Since some Japanese species of this genus exhibit few morphological differences, their classification has been confused. The Auplopus carbonarius species-complex has two morphological types in the male, i.e. the "whitely-spotted mandible type" and "black mandible type". The former has the mandible with a white spot and frons with lateral white bands extending above the level of the antennal socket, and the latter has the black mandible and frons with lateral white bands not extending above the level of the antennal sockets. The purpose of this study is to examine whether behavioral isolation exists between the both type wasps by experiments of copulation. Males and females were paired in petri dishes, and their behavior was recorded with a video camera. In the copulation between the same type wasps, the male moved toward the female with the metasoma raising and the wings vibrating, and then mounted on her. Next, the male inserted its aedeagus into her metasomal tip, when she stopped moving for a while. After beginning to move again, the female drove off the male that exhibited courtship behavior. Between the different type wasps, however, male courtship scarcely occurred and copulation was never successful. Differences in the mounting time and mating behavior were found between the both types. Based on the results, we concluded that behavioral isolation exists between the both types, and it is thus presumed with certainty that these have been differentiated to the species level.

Keywords:*Japanese Auplopus carbonarius species-complex, mating behavior, behavioral isolation, courtship behavior, copulation*

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PS1M031

Taxonomy

P1

The taxonomic study of the family Stygothrombiidae (Prostigmata:Acari)

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Stygothrombiid mites inhabit interstitices in mountain streams and lakes. However, relatively little has been known about the taxonomy and life-history of these mites. It is also difficult to identify stygothrombiid species because most of the descriptions and illustrations in the past studies do not provide adequate details for a meaningful comparison of the species. Therefore, the purpose of this study is to establish the classification of Stgothrombiidae on the basis of the re-examination of discriminating characters of species and genus. At first, we examined the suitability of generic and specific characters of Stygothrombiidae in past studies. As a result, several generic characters such as the number of setae in crista metopica, existence of a pattern in epidermis, existence of setae in claws, existance of setae in empodium, and the number of setae in glandularia were constant among genera. Therefore, these generic characters should be regarded as characters of familial rank. Although most of the specific characters in the past studies were valid, some additional discriminating characters were newly found. As a result of SEM observation, forms of glandularia on epidermis and odontus on palpi were varied among species. Consequently, these characters can be used for specific identification. We examined the classification of Stygothrombiidae based on the newly established characters. The five genera so far erected Cerberothrombium, Victathrombium, Charonothrombium, Hydorothrombium, Baicalacarus should be synonymized to a single genus Stygothrombium. Consequently, as indicated by Mullen and Vercammen-Grandjean (1980), all species of the stygothrombiid mites should belong to the single genus Stygothrombium.

Keywords:*Acari, Prostigmata, Stygothrombiidae, Classification*

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PS1M032

Taxonomy

P1

'Embioptera problem' in relation to an enigmatic taxon Timematodea

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The interordinal relationships within Polyneoptera are highly controversial probably because of ancient and rapid radiations. In order to circumvent the limitations of morphological classification, we sequenced the full length mitochondrial genome of Phasmatodea and Embioptera species and analyzed the molecular phylogeny of polyneopteran insects. There are advantages of mtDNAs for phylogenetic analyses, such as the absence of recombination, introns, and paralogs. PCR-based cloning of mtDNA is also helpful to obtain relatively large data sets. In the course of the analysis, we found novel gene rearrangements in a webspinner, Aposthonia japonica. The phylogenetic analyses showed that Embioptera forms a monophyletic clade with Verophasmatodea, a suborder of typical stick and leaf insects. Timema, which has been thought as an ancestral group of Phasmatodea, was placed as an outgroup of Embioptera + Verophasmatodea. There are discrepancies in intraordinal relationships of Verophasmatodea. For example, the molecular phylogeny of mitogenomes did not support the monophyly of Areolatae and Anareolatae, two major infraorders of Verophasmatodea. Mantophasmatodea and Grylloblattodea were closely related to Phasmatodea and Embioptera, forming a monophyletic clade. Dictyoptera, including the praying mantis, cockroach and termite, was placed as sister to the clade Phasmatodea + Embioptera. Orthoptera was recovered as a monophyletic group distantly related to Phasmatodea.

Keywords:*lower neoptera, stick insect, webspinner, Timema*

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PS1M033

Taxonomy

Taxonomic overview in marine water mites Pontarachnidae

Akira Moto[1], Hiroshi Abe[2]

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Pontarachnidae is the only family of the superfamily Hygrobatoidea in the subclass Acari occurring in the marine environment. This family contains two genera Pontarachna and Litarachna. Although the water mites of the family are widely distributed around the world, the taxonomic overview of the family has not been given since Viets, K. (1957). And in the former descriptions of the species in the family, the quantitative charcters has been used as major taxonomic characters. In this study, taxonomic characters of species were reconsidered by reviewing specific descriptions in the past studies as well as examining real specimens collected from the Pacific coast in Japan. As a result, it is concluded that most of the quantitative characters so far used in the specific descriptions were not suitable for discriminating taxonomic characters of species. On the other hands, configrations of glanduralia and platelets on the ventral side of body are considered as useful taxonomic characters. The critical review of the past study strongly suggests that Litarachna divergens is junior synonym of Litarachna communis and Litarachna curtipalpis is junior synonym of Litarachna sabangensis because these species were not divided by taxonomic characters newly established in this study.

Keywords:*taxonomy, Pontarachnidae, acari*

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PS1M034

Taxonomy

P1

Summary of four years of DNA barcoding on Pseudococcidae and their parasitoids

Thibaut Malausa[1], Jean-Francois Germain[2], Aleixandre Beltra[3], Margarita Correa[4], Aline Bertin[5], Shaaban Abd-Rabou[6], Hassan Shalaby[7], Ernesto Prado[8], Tamotsu Murai[9], Giuseppina Pellizzari[10], Emilio Guerrieri[11], Gerard Delvare[12], M. Bora Kaydan[13], Geraldine Groussier-Bout[14], Sylvie Warot[15], Aurelie Blin[16], Didier Crochard[17], Philippe Kreiter[18], Nicolas Ris[19]

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Since 2009 our teams have carried out DNA barcoding of mealybugs (Hemiptera: Pseudococcidae) and their parasitoids (mainly Hymenoptera: Encyrtidae). Mealybug populations and associated parasitoids have been surveyed on crops and ornamental plants from several regions of the world. Individual samples collected have been characterized by DNA sequencing at 2-5 DNA markers (using non-destructive DNA extraction methods) and then by morphological examination. Based on the characterisation of >3,000 samples, around 50 Pseudococcidae species and 30 parasitoid species have been barcoded and identified. These results make it possible to improve our knowledge of the systematics of these taxonomic groups and to develop diagnostic tools for pest management and biological control. However, many samples characterized by DNA sequencing are still in need of taxonomic identification. In addition, molecular data suggest that numerous taxonomic revisions and investigations on complexes of cryptic taxa are currently required.

Keywords:*Taxonomy, Phylogeny, Cytochrome oxidase, ITS, Pseudococcids, Scale insects, Platygastridae*

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PS1M035

Taxonomy

P1

A comparative survey of proboscis morphology in tiger moths (Lepidoptera: Erebidae: Arctiinae)

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Tiger moths are known for their vibrant coloration as well as their unusual feeding behaviors. In particular, some tiger moths sequester toxic compounds such as pyrrolizidine alkaloids, from their host plants for chemical protection as opposed to nutrition. This feeding behavior has been linked to mating behaviors and defense. While tiger moth behavior has been extensively studied, their morphology and evolution has not. This study documents proboscis chemosensory structures in adult tiger moths using scanning electron microscopy (SEM). SEM images of more than 40 species representing Arctiini were taken. Resulting images were used to describe proboscis morphology for all taxa included in this study. Adult pharmacophagous (PA feeders) species had a significantly higher number of chemosensory structures on the proboscis than species that are pharamcophagous as larvae. Putative olfactory sensilla are reported on the proboscis of Nyctemera coleta. Olfactory sensilla on the moth proboscis may play a role in sensing low-volatile plant secondary compound residues.

Keywords:*proboscis, morphology, Erebidae*

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PS1M036

Taxonomy

P1

Species of *Acizzia* (Hemiptera: Psyllidae) as potential new economic pests of solanaceous crops.

Gary Taylor[1], Deborah Kent[2], Alan Yen[3]

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Two species of Acizzia, A. alternata Kent & Taylor and A. solanicola Kent & Taylor (Hemiptera: Psyllidae) were recently described from the plant family Solanaceae from eastern Australia. Hosts were recorded as Solanum mauritianum Scop. (wild tobacco tree: host for both species) and S. melongena L. (eggplant: for the latter). As both host species were introduced into Australia, it was postulated that these psyllids may have either been accidental introductions from an unknown source, or of Australian origin that had switched hosts from native Australian solanaceous hosts. In 2011, the discovery of an undescribed species of Acizzia from native Solanum lasiophyllum Dunal ex Poiret (flannel bush) from Western Australia supported the hypothesis that this morphologically distinct 'species-group' within the Acizzia originated on Solanaceae within Australia. In early 2012, A. solanicola was recorded for the first time in New Zealand. In Australia, it is now considered a potentially serious pest species of eggplant. Nymphal feeding causes malformation of leaves, wilting, premature senescence and crop loss, although it is yet to be determined whether these symptoms are as a result of feeding alone, or of an undiagnosed transmissable pathogen. Its ability to transmit Candidatus Liberibacter solanacearum, (recorded in New Zealand, but not Australia) in other solanaceous crops (cape gooseberry, capsicum, chilli, tamarillo, potato and tomato) has yet to be assessed. The environmental implications of its potential role in the transmission of Candidatus, or other plant pathogens, in native Australian and New Zealand Solanaceae remains unknown.

Keywords:*Plant biosecurity, Candidatus Liberibacter solanacearum, Psyllid yellows*

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PS1M037

Taxonomy

P1

Genetic variation and phylogeographic inference of alpine ground beetle (Coleoptera, Carabidae, Nebriini) in Taiwan

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Taiwan is located off southeastern coast of China, between Ryukyu and Philippine archipelagoes. During the past two million years, Orogenesis events had produced over 200 peaks higher than 3000 meters in four mountain ranges separated by lower hills and canyons which become an important dispersal barrier for alpine insects. However, glaciations during Pleistocene may make populations moving down and migrating across lower elevation. We tested two glacial refugia hypotheses for alpine carabids in this study; one is nunatak refugia hypothesis which means alpine insects stay in situ in high elevation area and the other is migration hypothesis which has alternative refugia in southern or low elevated area. Phylogenetic structure among carabids in Nebriini based on two mitochondrial (COI and 16S) and two nuclear (wingless and 28S) sequences showed that Nebria niitakana, N. formosana and Leistus smetanai have great divergences in populations between mountain ranges. It means that these species stayed in situ in high mountains without migrations during the recent glaciation and supports nunatak hypothesis. However, L. nokoensis and N. uenoiana showed no population differentiation, and the fixation index and gene diversity data suggested the glacial refugia of these two species should be in the middle of Central Mountain Range or further south. The incongruent support of differentiation hypotheses among these species may be caused by different microhabitat requirements and migration ability, especially on N. uenoiana which evolved functional hind wings and may cover wilder habitats.

Keywords:*Mountain islands, Nunatak refugia hypothesis, Migration hypothesis, Nebria, Leistus*

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PS1M038

Taxonomy

P1

Morphological description of *Ptychocroca galenia* Brown & Razowski (Lepidoptera; Tortricidae) immature stages in laboratory conditions.

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Ptychocroca galenia Brown & Razowski (Lepidoptera: Tortricidae) is an endemic pest from Chile. It belongs to the recently described genus Ptychocroca, and the only information available for this species is the morphological description of the adult stage. The objective of this work was to study the morphology of immature stages. We describe the complete development of the progeny of an adult couple, reared in laboratory and fed with different substrates available in the area on which the adults were collected. Oviposition was in flatted, clumped eggs, with a completely reticulated chorion, in groups from two to five. From the first stage the larva presents a light brown head with a dark stripe slightly hinted to semi-complete in the gena area, brown protorax shield with a clearer divisor line at the center and a dark anal comb. A rough cuticle, with microspines and dark pinnacles in contrast with the rest of the body can be observed at the second larval stage. By the third larval stage the color of the larva is creamy, slightly pink. The pupa is described in details, being similar to others of the same family. Differences between this species pupa and others very similar are given. No inference about substrate preferences was possible. This study is the first made on P. galenia; it provides better morphological criteria for recognition of immature stages.

Keywords:*Ptychocroca galenia, immature, pupa*

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PS1M039

Taxonomy

P1

Revision of Western Japanese *Galloisiana* (Grylloblattodea): From a standpoint of female body characters

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In Japan, six species of the genus Galloisiana Gurney, 1948 have been reported. The Westernmost Japanese species, G. notabilis Silvestri, 1927, was described based on only one larva specimen and its description is lacking in information. Another Western Japanese species, G. chujoi Gurney, 1961, was reported based on an adult, but the described characters are insufficient for interspecific comparison. To revise Japanese Galloisiana species, detailed description of G. notabilis and G. chujoi based on adult specimens is necessary. Some specimens containing one adult female were collected from each type locality (Nagasaki Prefecture for G. notabilis and Kagawa Prefecture for G. chujoi) and studied in detail. It was revealed i) that the characteristics of the penultimate instar larva collected at the type locality of G. notabilis correspond to the description by Silvestri (1927), ii) that the females from the two type localities are similar in some important characters such as eyeless, small number of setae on the cervical sclerites, and the shapes of femur and tibia, suggesting that these two species should be synonymous with each other, and iii) that the above mentioned characters are clearly different in other four species of Japanese Galloisiana. To further the revision of the Galloisiana group, it is important to obtain male specimens of G. Notabilis and G. Chujoi, to identify new specimens recently collected from other habitats in Western Japan, and to compare Western Japanese species with Korean species.

Keywords:*Galloisiana notabilis, Galloisiana chujoi, Grylloblattodea, Japan, morphology*

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PS1M040

Taxonomy

P1

Taxonomic study of the genus *Altica* (Coleoptera: Chrysomelidae: Alticinae) from Japan, with emphasis on the morphology of the male and female genitalia, and mouth parts

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The flea-beetle genus Altica is widely distributed in all major biogeographic regions except the Antarctica. The genus Altica contains about 250 valid species, of which 13 species and one subspecies are known from Japan (Ohno, 1960; Kimoto and Takizawa, 1994; Lobl and Smetana, 2010). This genus includes agricultural pests such as A. fragariae, which feeds on strawberry leaves. Therefore, accurate identification of Altica species is essential for effective control of agricultural pest species. Nevertheless, it is difficult to identify Altica species based on the external morphology, because most of the species have similar external morphology and body color. It is suggested that Altica species can be identified mainly based on mandibles, the male genitalia, styli, speculum and spermatheca (e.g. Konstantinov, 1987; Kangas and Rutanen, 1993; LeSage, 1995). Although the male genitalia have been studied for Japanese species of the genus Altica, mandibles and female genitalia have not been well described. Thus, female specimens of Altica were sometimes misidentified, and there are some taxonomic confusions. In the present study, I taxonomically revised the genus Altica in Japan, with special emphasis on the morphology of mandibles, male genitalia, styli, spiclum, and spermatheca. As a result, a total of 15 species and one subspecies are recognized from Japan. These species can be reliably distinguished not only by the male genitalia as suggested by previous studies, but also by mandibles and female genitalia.

Keywords:*taxonomy, morphology, aedeagus, SEM, Altica, Japan*

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PS1M041

Taxonomy

P1

The morphology of the apical portion of abdomen in the Genus *Malthodes* (Coleoptera: Cantharidae): Search for taxonomic characters in male and female

Naoki Takahashi
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The genus Malthodes is characterized by having the 9th abdominal sternite more or less well developed in the male and this feature is useful for species identification though the sternite is sometimes similar in general appearance among the species. In marked contrast to the male, females are often very similar to one another and indistinguishable by external morphology. Therefore, identification based upon female characters has seldom been attempted. Twenty-five species of the genus Malthodes have been known from Japan, but many undescribed congeners have been collected from various localities in recent years and female material remained undetermined. In the course of my revisional study of this genus in Japan, the males of 24 species and the females of 15 species including five undescribed species on each sex were examined, and then the developed 10th tergites in the males or the depressed abdominal tergites in the females ["copulatory depression" (Takahashi, 2001)] were found in some congeners. In addition, some differences between the female congeners in the shape of terminalia were recognized. These characters are regarded as the possible key characters for identifying species in the genus, particularly in the female.

Keywords:*taxonomy, Malthininae, Malthodini, copulatory depression*

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PS1M042

Taxonomy

P1

Rediscovery of Ludlow's Bhutan Glory, *Bhutanitis ludlowi* Gabriel (Lepidoptera: Papilionidae): Morphology, biology and biogeography

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The papilionid butterfly, Bhutanitis ludlowi Gabriel, 1942, was discovered from Bhutan in 1933 and 1934. Since its discovery many researchers and collectors have attempted to rediscover this mysterious swallowtail but without success. In August 2009 and 2011, we rediscovered this species after a lapse of 76 years since it was first collected in Trashiyangtse Valley of Trashi Yangtse, eastern Bhutan. At the same time, the immature stages of this butterfly were recorded for the first time. In this presentation, we describe and illustrate morphological and biological features of B. ludlowi based on observations of the adult, egg and larval stages. The most noteworthy feature is its oviposition strategy. This species lays its eggs in a mound composed of about 100-180 eggs. As far as we are aware, this type of egg-laying behavior has not been recorded in the Papilionidae. We show the affinities to and differences from the other three Bhutanitis species, in particular the closely related B. lidderdalii Atkinson. We also briefly discuss its habitat, conservation needs and biogeography.

Keywords:*Bhutan, conservation, early stages, eggs, endemic species, hostplant*

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PS1M043

Taxonomy

P1

World Larainae (Coleoptera: Elmidae) - Taxonomy, phylogeny and distribution

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The subfamily Larainae includes 28 genera and around 130 species. The group is distributed worldwide, however the greatest species diversity is in the Afrotropical and Neotropical regions. Larainae represents distinct group of semiaquatic beetles within family Elmidae, distinguishing by many morphological and ecological features. The taxonomy and phylogeny of the group is however still very poorly known. For reconstrucion of the phylogeny and taxonomy of Larainae we used several mitochondrial and nuclear DNA fragments. For the analysis, the samples representing about half of the world genera were available (the largest collection of Larainae samples usable for DNA analysis). We have tested monophyly of the group, relationships with other Elmidae genera and we also assessed the phylogenetic position of several genera with deviated morphological features. Based on the latest information, the updated checklist of genera is provided including notes on the world distribution of the subfamily. Beside population study, the data on intraspecific variability will serve for development of determination technology of animal species using DNA. This contribution/publication is the result of project implementation (ITMS: 26240220049) supported by the R&D OP funded by the ERDF.

Keywords:*Coleoptera, Elmidae, Larainae, taxonomy, checklist, DNA*

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Taxonomy

P1

The preliminary phylogeny of the Epicopeiidae (Lepidoptera, Geometroidea)

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The Epicopeiidae comprises about only 20 species endemic to the eastern Palaearctic and Oriental regions. Adults of this family are conspicuous with variable wing shapes and involve mimicry with many lepidopteran groups, such as Papilionidae, Riodinidae, Nymphalidae, Geometridae, and Lymantriidae. The family was previously only consist of the type genus Epicopeia. The remaining genera such as Amana, Chatamla, Parabraxas, Psychotrophia, Schistomitra, Deuveia and Burmeia were subsequently transferred to this family from an older concept of Epipleminae (Uraniidae). The family was associated with Drepanidae for a long time until recent studies prove its affinity with Geometridae and Uraniidae. The internal relationships of the Epicopeiidae were only assessed using morphological characters and limited taxa and a hypothetical outgroup. In the present study, we reconstructed the first phylogenetic hypothesis of this peculiar family using nucleotide sequence information from two genes, COI and Ef1a, and Bayesian analyses. The preliminary results show that the family is monophyletic, while some genera may not be monophyletic and it suggests that the mimicry with butterflies may have evolved independently in different lineages and some cryptic species are revealed by the present study.

Keywords:*mimicry, Geometridae, Epicopeiidae, Uraniidae, wax, Oriental region*

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Taxonomy

P1

Asian Species Information Databases KONCHU and AIIC Based on Asian Insects

Osamu Tadauchi
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Publicly available entomology databases KONCHU and AIIC (Asian Insect Information Center database) were constructed. The former is a general database name which includes various files of bibliographical, image and inventorial databases. The bibliographical database, KONCHUR, is a taxon-based entomology database based on the main Japanese and Pacific entomological journals (24 journals (= files) at present). It includes many East Asian and Pacific as well as Japanese insects. The image database includes useful Japanese insects, bees and encyrtid parasitic wasps with images. The inventorial database, MOKUROKU (Check list of Japanese insects) and DJI (Dictionary of Japanese insect names) cover all the Japanese insect names. The latter, AIIC, is aimed for the accumulation of insect data of type specimens and normal specimens deposited in universities, institutions and museums in Japan. Each record was written according to the format of Darwin Core of the Global Biodiversity Information Facility (GBIF). The records in KONCHU and AIIC are written mainly in English. It is administered by a SIGMA management system. Outlines of the KONCHU and AIIC are presented. A user can access the database files by visiting the following URLs: http://konchudb.agr.agr.kyushu-u.ac.jp/ and http://aiic.jp/.

Keywords:*bioinformatics, Asian Insect Information, KONCHU, AIIC, SIGMA.*

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Taxonomy

P1

Status of Phalangopsidae (Orthoptera, Grylloidea) from the atlantic forest of Brazil: Diversity, distribution and founds of recent expeditions

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Phalangopsid crickets are known as long-legged or spider-like crickets due its large development of posterior legs. Besides, the large scape, the shape of head, palpi, tibial spurs and male genitalia comprises the main characters used to define this taxon. Phalangopsidae currently includes 903 species in the world and is a well distributed cricket's family in Neotropics, being the most common on the understories of tropical forests; its species inhabits the leaf litter, trees, trunks, barks and any kind of natural cavities, including caves. In Brazil the phalangopsid's fauna comprises 31 genera and 76 species in three subfamilies (Luzarinae, Phalangopsinae and Paragryllinae). Although phalangopsid species are distributed throughout Brazilian biomes, mainly on tropical forests, its diversity is poorly known: only 8 species are cited from Brazilian Amazonia and 40 from Atlantic Forest. This second one is a tropical forest occurring along the atlantic coast and now considered a biodiversity hotspot due it's great number of endemic species and fragmentation; only 7% of this forest is preserved. The Brazilian studies on taxonomy of crickets are still unsatisfactory in comparison with the extant diversity: only 24 species were described over the last 10 years. Here it will be presented maps of distribution of Phalangopsidae in Brazilian territory, results of recently expeditions through the Atlantic Forest and the presentation of several new species found. It's expected that with efforts on field works and formation of specialists, the number of cricket species will greatly increase, expanding our knowledge on Neotropical cricket's fauna.

Keywords:*Grylloidea, Phalangopsidae, biodiversity, crickets*

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PS1M047

Taxonomy

P1

Phylogeny of the neotropical social wasp *Synoeca* (Hymenoptera, Vespidae, Polistinae)

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Synoeca is a genus of Neotropical social wasps with five species. Phylogenetic studies based on morphological characters have been controversial because of the polymorphic nature of some phenotypic characters used. In the present work we combine molecular and morphology to perform a phylogenetic analysis of the group. Specimens of the five species and outgroups were collected and preserved in ethanol 100%. Standard molecular techniques were performed to extract, amplify and sequence genetic data from the mitochondrial (cytochrome oxidase I, 12S and 16S) and nuclear (28S, histone 3 and elongation factor 1 alpha) genomes. The sequences were analyzed and the alignment was performed using the software MUSCLE. The gathered data totalizes 5279 characters for eight species. The searches for the best tree were performed using the software TNT where a strategy of combined algorithms was used. Resampling analyses were performed originating Jackknife absolute frequencies and GC values. We found 1 most parsimonious trees with 646 steps, ci 88, ri 72, showing the relationship (Protopolybia (Charterginus (Metapolybia ((Synoeca virginea, Synoeca chalibea), (Synoeca septentrionalis (Synoeca cyanea , Synoeca surinama))))). These results support previous works where polymorphic morphological characters are not taken into account for phylogenetic studies.

Keywords:*Epiponini, Social wasps, Swarm founders*

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PS1M048

Taxonomy

P1

Ants diversity on agroecosystems in the Tak Province, Thailand

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The present study aims to see diversity of ants between land use types in agroecosystems in the Tak Province of Northern Thailand. A survey was conducted to determine the species of ants in the agroecosystems at the Center of Agricultural and Development. Ants were collected in two locations, tea orchard and avocado orchard. Three sampling methods namely honey bait-traps, cheese bait-traps and pit fall traps were used. A total of 26 species belonging to four subfamilies of ants were collected. The subfamily Myrmicinae had 11 species, Ponerinae 9 species, Formicinae 5 species and Dolichoderinae 1 species. The tea orchard collected 17 species with the dominant genus, Crematogaster, trapped from honey-bait traps and cheese bait-traps. The pit fall traps collected Anoplolepis gracilipes Smith The genuses found in the tea orchard were Aphenogaster, Crematogaster, Polyrhachis and Oecophylla. The avocado orchard collected 16 species with Dolichoderus as the dominant genus from honey bait-traps and cheese bait-traps. Paratrechina longicornis and Pheidole sp.1 were found in the pit fall traps. Three Genuses found in avocado orchard were Amblyopone, Dolichoderus and Paratrechina.

Keywords:*Ants diversity, agroecosystem*

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Taxonomy

P1

Three new species of the genus *Hylaeus* (Hymenoptera: Colletidae) from South Korea

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Three new species, Hylaeus (Nesoprosopis) dathei sp. nov., H. (Nesoprosopis) jirisanensis sp. nov. and H. (Nesoprosopis) koreaensis sp. nov. of the genus Hylaeus are described from South Korea. The diagnosis of each species is provided. Illustrations of male genitalia for these new species are also included.

Keywords:*new species, South Korea, Hylaeus, Nesoprosopis*

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Taxonomy

P1

Revision and phylogeny of Chinese *Phaedon latreille* and related genera (Coleoptera: Chrysomelidae: Chrysomelinae)

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The leaf beetle genus Phaedon Latreille, 1829 from China are revised and illustrated. A key to the Chinese species, a generic redescription, distributional records, the natural history of the genus including the host plants records are provided. The phylogeny based on 50 exoskeletal and genitalic characters from six genera as ingroup (Neophaedon Jakobson, 1901, Phaedon, Prasocuris Latreille, 1802, Sclerophaedon Weise, 1882, Sternoplatys Motschulsky, 1860, Yunnaedon Daccordi & Medvedev, 1999) and one genus (Timarcha Latreille, 1829) as outgroup suggests a clade comprising Neophaedon and Phaedon, another clade comprising Yunnaedon and Sclerophaedon as the monophyly groups. The strict consensus tree of the analysis is not well resolved by both PAUP and Winclada. Nevertheless, considering the results of the initial analysis and the apparent instability of the phylogenetic results based on morphological data alone, we decided to maintain the generic rank of all genera at this stage. A biogeographic analysis for Chinese Phaedon is also discussed. Whereas only Phaedon brassica Baly, 1874, P. cochleariae Fabricius, 1792, P. armoraciae Linnaeus, 1758 are restricted to relatively low altitude, the other species show a major radiation in the Hengduan Mountain Region above 2800 m, which is presently the center of diversity of the genus. Most of these species are flightless and show a preference for scree slopes and rock crevices.

Keywords:*Phylogeny, Phaedon, Chrysomelinae, Chrysomelidae*

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PS1M051

Taxonomy

P1

Taxonomic revision of the family Issidae from Korea (Homoptera: Fulgoromorpha)

Mohammad Atikur Rahman[1], Yong Jung Kwon[2], Eun Yeop Huh[3]

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The planthopper family Issidae sensu stricto, one of the smaller fulgoroid families in Korea, is revised taxonomically. Four genera and five species have been recorded in the Korean fauna. Among them, one genus, Sivaloka Distant with a species, S. damnosus chou et Lu, is new to Korea, and a new species Gergithoides jejudoensis sp. nov., that was previously misidentified as Gergithoides carinatifrons Schumacher from Korea, is herein described with illustrations. Key to tribe, genus and species from Korea are provided.

Keywords:*Auchenorrhyncha, Fulgoroidea, Gergithoides jejudoensis, Planthopper, Sivaloka*

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PS1M052

Taxonomy

P1

Taxonomic revision of the family Tettigometridae from Korea (Homoptera: Fulgoromorpha)

Mohammad Atikur Rahman[1], Yong Jung Kwon[2], Sang Jae Suh[3]

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The planthopper family Tettigometridae, one of the smaller fulgoroid families in Korea, is revised taxonomically. In the revision, three species are confirmed and one species, Tettigometra (Mimarada) bipunctata Matsumura, is unavailable in our current investigation. Among three species, Tettigometra (Tettigometra) burjata Kusnezov, 1929 is new to Korea, and Tettigometra (Macrometrina) sobaeksana sp. nov. is new to science. Previously recorded Tettigometra (Macrometrina) grossa Lindberg in the Korean checklist is treated Tettigometra (Macrometrina) fusca (Melichar, 1902) herein following the description of Emeljanov (1980). All species are described and illustrated, and identification key to subgenera and species from Korea are provided.

Keywords:*Planthopper, Auchenorrhyncha, new taxa, taxonomy, Korea.*

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PS1M053

Taxonomy

P1

A molecular phylogenetic study of the Korean Omaliinae MacLeay (Coleoptera: Staphylinidae)

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A phylogenetic analysis of the Korean Omaliinae MacLeay is presented based on molecular characters. The data set comprised partial mitochondrial COI (1501 bp), COII (372 bp), 12S rDNA (351?356 bp), and nearly complete sequences of 18S rDNA (1852?1862 bp) for 20 species. Nineteen omaliine species in 12 genera and four outgroups were included. The sequences were analyzed simultaneously by parsimony, maximum likelihood (ML), and partitioned Bayesian analysis for the combined data. In all analyses, the tribe Eusphalerini Hatch were supported as being monophyletic (clade A) and the genus Geodromicus Redtenbacher formed a clade with the tribe Eusphalerini. Also, Omaliini MacLeay (clade B), and Camioleum choi Shin and Ahn and Olophrum sp.1 showed a sister group relationship (clade C). The tribe Coryphiini Jakobson was monophyletic in two analyses (clade D). The genus Uenohadesina Smetana was a sister group to Trigonodemus koreanus Kim and Ahn in parsimony analysis (clade E), but it formed a clade with Camioleum choi and Olophrum sp.1 (clade F) by the other two analyses.

Keywords:*Molecular Phylogeny, Coleoptera, Staphylinidae, Omaliinae, Korea*

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Taxonomy

P1

Generic review of the tribe Athetini (Coleoptera: Staphylinidae: Aleocharinae) in Korea

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The Athetini Casey, containing thousands of species in 219 genera, are the largest and the most taxonomically difficult tribe in the Aleocharinae. They are dominant predators in diverse microhabitats and undoubtedly the most successful small beetles. In spite of their taxonomical and ecological diversity, members of Athetini are poorly characterized, but in general, they are distinguished from other aleocharine genera by combination of the following characters: coeloconic sensilla on antenna absent; frontal suture absent; maxilla with four palpomeres; labium with three palpomeres; tarsal formula 4-5-5; athetine bridge of the aedeagus present. In the Palearctic, 1931 species have been recorded in 90 genera. In Korea, Bernhauer was the first entomologist who reported one athetine species in 1923. Later, Pa?nik (2001) presented a taxonomic study including 82 species in 22 genera from North Korea. Currently, a total of 90 species in 23 genera has been recorded in the Korean peninsula. Five genera are discovered for first time from the Korean peninsula (Earota Mulsant & Rey, Enalodroma Thomson, Osakatheta Maruyama et al., Saphocallus Sharp, and Thamiaraea Thomson). As a result, we recognize 28 athetine genera from the Korean fauna representing about 30% of the Palearctic fauna.

Keywords:*Coleoptera, Staphylinidae, Aleocharinae, Athetini, Korea, Taxonomy*

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PS1M055

Taxonomy

P1

Phylogeny of the subtribe Homalotina Heer (Coleoptera: Staphylinidae: Aleocharinae)

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A cladistic analysis of the subtribe Homalotina Heer is presented. Seventeen homalotine species in nine genera and eleven outgroups are included. The subtribe is hypothesized to be a monophyletic group consisting of nine genera (Anomognathus Solier, Cephaloxynum Bernhauer, Holisomimus Cameron, Homalota Mannerheim, Homalotina gen. nov., Neomalota Cameron, Paractocharis Cameron, Stenomastax Cameron, Thecturota Casey) based on following synapomorphies: body dorsoventrally strongly flattened, head as wide as pronotum, and antennae inserted anterior to a line drawn between anterior margins of eyes. A history of the classification of the Homalotina is discussed. The data set for phylogenetic analysis comprised 113 characters representing 371 character states derived from adult morphology.

Keywords:*Coleoptera, Homalotina, monophyly, morphology, phylogeny, Staphylinidae, synapomorphy*

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PS1M056

Taxonomy

P1

A review of the coastal Staphylinidae (Coleoptera) in Korea

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The family Staphylinidae are one of the most diverse beetle families containing about 55,440 species in 32 subfamilies from the world. In Korea, 557 species in 15 subfamilies have been recorded. They are found nearly all habitats on earth, such as forest, riverside, alpine region, cave, seashore, and so on. Frank and Ahn reported 392 staphylinid species in 92 genera confined to seashore habitats (include intertidal and splash zone, but exclude coastal sand dune) worldwide. In Korea, a total of 36 species in 18 genera has been known from various microhabitats (e.g. under seaweeds/seagrasses, under stones, under debris, empty barnacles/shells etc.) of coastal environs, since Lobl described Briara palpalis (= Prosthecarthron sauteri Raffray) from North Korea in 1974. Species diversity of the Korean coastal rove beetles comprises about 9.2 % of worldwide and they represent four subfamilies (one in Pselaphinae, 23 in Aleocharinae, two in Paederinae, 10 in Staphylininae). Here, we investigated the diversity of Korean coastal species in terms of species numbers among countries, taxa (subfamily level) and microhabitats.

Keywords:*coastal, Coleoptera, diversity, fauna, Korea, Staphylinidae*

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PS1M057

Taxonomy

P1

Diversity of diving beetles (Coleoptera: Dytiscidae) in Korea

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Diversity of Diving Beetles (Coleoptera: Dytiscidae) in Korea Dae-Hyun Lee and Kee-Jeong Ahn Department of Biology, Chungnam National University, Daejeon, Korea The family Dytiscidae are the most diverse family among water beetle families and live in nearly every aquatic freshwater ecosystem. Members of the family Dytiscidae are characterized by combination of following characters: body shape streamlined; antenna with 11 antennomeres and filiform; metacoxa anteriorly strongly expanded; metaventrite without transverse suture and beak shaped on lateral parts; metacoxal process with large outer lobes; simultaneous hind leg movements. The Dytiscidae contain about 4,100 species in 190 genera (27 tribes, 10 subfamilies) from the world. In Korea, 59 species in 20 genera (13 tribes, 6 subfamilies) have been recorded; 279 species in China, 112 species in Japan, 51 species in Taiwan and 97 species in Far East of Russia. In 1886, Kolbe first discovered four species from Korea. Later, Mori (1932) reported 27 species including one new species. Cho was the first Korean beetle taxonomist who described 24 species in 1969. Since then, about 15 Korean entomologists and a few foreign entomologists have been adding 35 dytiscid beetle species to the Korean fauna.

Keywords:*Diversity, Dytiscidae, Korea*

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PS1M058

Taxonomy

P1

Korean species of the genus Lordithon Thomson (Coleoptera: Staphylinidae: Tachyporinae)

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The genus Lordithon Thomson contains 134 species and distributed worldwide. In Korea, two species (L. bicolor Gravenhorst, L. simplex Sharp) have been recorded. Members of the genus Lordithon are characterized by the combination of the following characters: body narrowly fusiform, strongly narrowed from elytral apex to both front of head and anal end; rather convex; surface glossy; elytra usually yellowish-red to reddish-brown with dark brown to black maculations. In this paper, we report five Lordithon species, three of which are identified for the first time in Korea (L. aitai Li & N. Ohbayashi, L. ohbayashii Li & Zhao, L. semirufus Sharp). A key, diagnoses, habitus photographs and line drawings of diagnostic characters are provided.

Keywords:*Lordithon Tachyporinae Staphylinidae Korea*

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PS1M059

Taxonomy

P1

A taxonomic review of the subfamily Anteoninae (Hymneoptera: Chrysidoidea: Dryinidae) from South Korea, with description of a new Species

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The subfamily Anteoninae R.C.L. Perkin, one of the largest subfamilies within the family Dryinidae, consists of 502 extant species in five extant genera: Anteon Jurine, 1807; Deinodryinus R.C.L. Perkins, 1907; Lonchodryinus Kieffer, 1905; Metanteon Olmi, 1984; Prioranteon Olmi, 1984 around the world. And, there are 44 species in four genera recorded from the Palaearctic region. Until now, only one genera and four species of Anteoninae, Anteon koreana, Anteon javanum, Anteon jurineanum and Anteon munitum, have been reported in South Korea. Members of the Anteoninae are the ectoparasitoids of Cicadellidae (Hemiptera: Auchenorrhyncha). Most species of the subfamily are important in natural control of agricultural. Types are all deposited in Yeungnam University, South Korea. The new and newly reordered taxa are listed as follows: A new species, Anteon sp. nov. and eleven unrecorded species, Anteon achterberi Olmi, 1989; Anteon ephippiger (Dalman, 1818); Anteon esakii Yasumatsu, 1960; Anteon flavicorne (Dalman, 1818); Anteon insertum Olmi, 1989; Anteon medium Olmi, 1997; Anteon pilosum Xu, Olmi & He, 2010; Anteon reticulatum Kieffer, 1905; Anteon septentrionale Xu, He & Olmi, 2002; Anteon takenoi Olmi, 1995; Lonchodryinus infuscatus Xu, Olmi & He, 2009. Additionally, Anteon medium from China and Anteon esakii from Russia are recorded for the first time. In this study, the sixteen South Korean species of Anteoninae are reviewed, and one new species, Anteon sp. nov. Kim and Lee are described. A key to the South Korean species, the illustrations and photographs of diagnostic characters are provided herein.

Keywords:*Anteon, Lonchodryinus, new speices, South Korea*

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PS1M060

Taxonomy

The distribution of *Autocrates maqueti* (Coleoptera: Trictenotomidae) in Korea

Jinyoung Park[1], Kyung Ho Kim[2], Seong Joon Park[3], Jong Kyun Park[4], Jong Eun Lee[5], Jin Young Park[6], Myung Jin Kim[7]

[1]Kyungpook National University, Republic of Korea, [2]Yeongyang-gun, Republic of Korea, [3]National Institute of Environmental Research, Republic of Korea, [4]Kyungpook National University, Republic of Korea, [5]Andong National University, Republic of Korea, [6]National Institute of Environmental Research, Republic of Korea, [7]National Institute of Environmental Research, Republic of Korea

The genus Autocrates Thomson, 1860 is currently represented by four species in the world fauna, namely A. aeneus (Westwood, 1846), A. maqueti Drumont, 2006, A. oberthueri Vuillet, 1910 and A. vitalisi Vuillet, 1912. Two of them, A. aeneus (Westwood) and A. vitalisi Vuillet, are widely distributed in the Oriental and South-East Palaearctic regions, while A. oberthueri Vuille, is limited to South China, and A. maqueti has been recently described from central and South China. New species to the fauna of the Korean peninsula; the most North-eastern record for the genus Autocrates. This species has longer been misidentified as aberrant A. aeneus, but after A. maqueti has been described (Drumont, 2006), status of Korean specimen has been completely clarified. Lin et al. (2008) presented review on distribution of Autocrates in China with notes on general distribution of each species. The most northern record for the genus Autocrates recorded before was a record of A. maqueti from Central China. The current record of the Korean A. maqueti is the most eastern and northern known record for this species and for the genus Autocrates in general. General distribuction of this species is still unclear. So far, we introduce of the investigation the state of distribution of A. maqueti in Korea. After more thorough investigation of its ecological data. Also, for this species we will conduct ongoing monitoring. This study was supported by ME & NIER (Survey on Ecosystem and Landscape Conservation Areas)

Keywords:*Coleoptera, Trictenotomidae, Autocrates maqueti, distribution, Korea*

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PS1M061

Taxonomy

A preliminary report of the Family Therevidae in Korea (Diptera: Asiloidea).

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The family Therevidae is one of the large families of Brachycera flies with 99 recognized genera and about 1,600 described species in the world. They are known to occur in all the zoogeographical regions. They prefer bushy edges of woods, often visibly alight in sunny patches on dry sandy areas, rocks, or trunks of trees. They can fly very fast for a short distance. Some larvae are known as leaf mould and humus eaters. Some species can be found under rotten tree bark and in decaying fruit. The family can be distinguished from the other families of Asiloidea by the following characteristics: 1) antennae with long scape, short pedicel and elongated flagellomere 1, 2) head almost hemispherical and not excavated at vertex, 3) slender to moderately thick body, 4) abdomen with eight well developed pregenital segments, 5) wing venation usually without vein $M_3$and 6) the costa extending around the entire wing. In Korea, there are five previously recorded species. As a result of ongoing investigation of the Korea Therevidae, we have so far recognized 15 species.

Keywords:*Asiloidea, Therevidae*

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PS1M062

Taxonomy

P1

Taxonomic review of the genus *Epistrophe* (Diptera: Syrphidae) in Korea

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[1]Yonsei University, Republic of Korea, [2]21stC Life Science Foundation, Republic of Korea, [3]Yonsei University, Republic of Korea

The genus Epistrophe is a relatively large genus with 51 recognized species of predominantly Palaearctic distribution (40 species). There are also 7 Oriental, 8 Nearctic and 1 Australian species. They can be distinguished from other syrphid genera by combination of the following characteristics: 1) scutum usually shiny to subshiny black with yellow pruinosity laterally; 2) anterior anepisternum, anepimeron, meron, and metapleuron bare; and 3) katepisternum with dorsal and ventral pile patches narrowly joined posteriorly (subgenus Epistrophe) or broadly separated (subgenus Epistrophella). In Korea, only three species have been previously recorded. In the present study, we have recognized the following seventeen Korean Epistrophe species: 1) eight possible new species: E. n. sp. A to H; 2) six new to Korea: E. aeka Kimura, E. aino (Matsumura), E. annulitarsis (Stackelberg), E. griseofasciata (Matsumura), E. sasayamana (Matsumura), E. shibakawae (Matsumura); and 3) three previously recorded Korean species: E. flava Doczkal and Schmid, E. grossulariae (Meigen), E. nitidicollis (Meigen). We here provide a key and photographs with their key characters indicated.

Keywords:*Epistrophe, Syrphidae*

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PS1M063

Taxonomy

P1

A preliminary list of the family Tachinidae (Diptera) in Korea

Han-saem Lee[1], Hye-woo Byun[2], Hyun-suk Lee[3], Jong-su Lim[4], Dong-jun Cha[5], Ho-yeon Han[6]

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The family Tachinidae is the second largest family of Diptera with approximately 10,000 described species and many thousands of undescribed species world wide. Most tachinid flies are noticeably bristled, but their body shape and size vary across the family. In addition to the features shared with other Oestroidea, adult tachinids possess a well-developed subscutellum, a rounded bulge below the thoracic dorsum. As tachinids are internal parasitoids of other arthropods (mostly insects) in their larval stage, they are considered to be aeconomically important group as natural enemies against agricultural and forestal pests. Tachinids employ direct or indirect strategies in attacking their hosts. Despite their economic and ecological importance, relatively little research has been conducted. In Korea, 104 species under 63 genera of Tachinidae have been previously recorded with many suspicious identifications. As an on-going systematic study of Korean Tachinidae, we have so far identified 150 genera and 216 species by comparing with the type specimens or original and subsequent descriptions. We here provide a preliminary list of Korean Tachinidae.

Keywords:*Tachinidae, Diptera*

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Taxonomy

P1

New records of genus *Hyphasis* (Coleoptera: Chrysomelidae: Alticinae) from Korea

Mi Hyun Kang[1], Jong Eun Lee[2]

[1]*Andong National University, Republic of Korea,* [2]*Andong National University, Republic of Korea*

The Alticine genus Hyphasis Harold, 1887 and a species of this genus H. parvula Jacoby, 1884 are recorded from Korea for the first time. The species of the genus Hyphasis Harold is distributed in the South eastern of Palearctic and Oriental region and more than 40 species were recorded in the world. The genus Hyphasis can be distinguished from the other genera by combination of following characteristics: pronotum without any ante-basal transverse impression; posterior tibia with a pointed spine at the apex; claw-segment of hind tarsus strongly dilated. The species H. parvula is easily distinguished from the published Hyphasis species by the following characters: body reddish brown; vertex impunctate, frontal ridge very thin and low anteriorly, slightly widened antennal calli; antennae blackish brown except for two basal segments, second segment shorter than third; elytra without a patch, epipleura extraordinarily broad, confusedly punctate.

Keywords:*new records, Hyphasis, Alticinae, chrysomelidae*

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PS1M065

Taxonomy

P1

Comparative analysis of *Dolichoderus sibiricus* Emery (Hymenoptera : Formicidae) between Korea and Japan

Eui Jeong Hong[1], Min Jee Kim[2], Ki-gyoung Kim[3], Iksoo Kim[4], Byung-jin Kim[5]

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We conducted a comparative analysis to know the intra-specific variations among Dolichoderus sibiricus Emery collected from Korea and Japan. The D. sibiricus collected from three Korean regions and one Japanese region (Hokkaido) were compared by morphological measurement and DNA sequence analysis of 658-bp of mitochondrial barcode region. The morphological measurements are as follows (mm): Japanese; AL 1.193, TL 3.885, CI 104.1, SI 81.85, EI 23.95, Cheongyang; AL 1.216, TL 4.015, CI 105.3, SI 80.97, EI 25.64, Hongcheon; AL 1.017, TL 3.609, CI 106.1, SI 82.1, EI 24.18 and Uijeongbu; AL 0.985, TL 3.228, CI 106.1, SI 80.9, EI 24.71. The morphological analysis has shown difference in the 4 Regions. Although specimens from Uijeongbu and Hongcheon were similar, those from Cheongyang were different. On the other hand, specimens from Japan and Cheongyang were similar, but the form of mesonotum and propodeum differ between them. The results of the COI analysis showed sequence variation, ranging from 0.15% (1 bp) to 8.2% (54 bp). The Korean specimens from three regions showed as large as 0.3%, whereas comparisons to Japanese specimens showed 8.2% (54 bp). These results collectively suggest that at least Korean and Japanese specimens differ to each other, indicating intra-specific variations between Korea and Japan. Considering current data were obtained from limited sample size further conclusive result can be obtained as more specimens are analyzed.

Keywords:*Dolichoderus, Formicidae, COI, Korea, Japan*

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Taxonomy

P1

Taxonomic revision of the leafhopper genus *Macropsis* Lewis from Korea (Homoptera: Cicadellidae)

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The genus Macropsis Lewis is revised taxonomically from Korea, primarily based on the abdominal apodemes, male genitalia, and 2nd valvulae of ovipositor. Previously only three species were erroneously recorded from Korea. Among them, Macropsis prasina (Boheman, 1852), has been misidentified with Macropsis notata (Prohaska, 1923) that is mentioned here. The other two species, Macropsis scutellata (Boheman, 1845) and Macropsis illota (Horvath, 1899), are declared absent in Korean fauna because of their superficial resemblance with allied species. A total of 7 species are recognized in this revision, among which six are new to science. All the species are described and illustrated, and identification key to species from Korea is provided.

Keywords:*Auchenorrhyncha, leafhopper, Macropsis, new species, taxonomy, Korea.*

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PS1M067

Taxonomy

P1

A checklist of the collembolan species from Korea

Ji Sup Kwak[1], Sangkyu Kang[2], Kyung-Hwa PARK[3]

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The Korean Peninsula is located between 33˚ and 43˚ north latitude, and 124˚ and 131˚ east longitude. Korea's total land area is 100,033 sq km including North Korea, and mountains cover 70% of Korea's land mass. The high mountain ranges with its complicated topographies, naturally, contain a variety of environments and include subtropical, temperate, alpine forests areas. Korea also has more 3,000 islands that surround the peninsula. As these complicated geography, the Korean Peninsula consists of several ecoregions. Collembola are small, primitive soil insects and their habitat varies greatly. They are ubiquitous, being found in all terrestrial biomes, including mountain tops, polar regions and deserts. As a result of its world-wide distribution, approximately 8000 species have been reported throughout the world (Janssens, 2012). The first recognizable Korean Collembolan species were two species listed by Kinoshita (1932): Tomocerus varius Folsom and Bourletiella hortensis Fitch. Many species of this group have been reported subsequently from the Korean Peninsula since then and now 255 species (including North Korean species) in 85 genera belonging to 18 different families are reported in Korea based on literature records.

Keywords:*Collembola, Checklist, Korea*

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PS1M068

Taxonomy

P1

A checklist of the Korean aquatic Collembola

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Waltz and McCafferty (1979) divided Collembola into three categories based on the relative degree of association and adaptation to the aquatic environment. Primary aquatic associates are those found exclusively in aquatic habitats; secondary aquatic associates are found in and around aquatic habitats, but a also inhabit other areas with high humidity and moisture; and tertiary aquatic associates, typical in part of the transitory neuston and inhabiting temporary pools formed by rain while having the least apparent adaptation to aquatic habitats. A number of systematic studies of hydrophilous Collembola on seashores and associated with freshwater have been carried out in North America, Japan, and several European countries. However, the Korean aquatic Collembolan fauna has still been very poorly investigated. The only report from Korean freshwater habitats was that of Kim and Lee (2000) listing ten species in nine genera, of which four species were new to science and six were new records for the Korean Peninsula. For our intensive study on halophilous Collembolan fauna in Korea, we scanned printed records and laboratory collections of collembolan species based on materials from freshwater. A total of 28 species in 19 genera belonging to 9 different families are reported in Korea.

Keywords:*halophilous, aquatic Collembola, checklist, Korea*

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PS1M069

Taxonomy

P1

Nematode identification of imported agricultural crops in plant quarantine of Republic of Korea

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Diagnosis of plant quarantine species is important in the process of the importation of the exotic plants and agricultural products from various foreign countries. There have been difficulties for the diagnosis of exact nematode species in plant quarantine due to the deficit of the information of exotic nematode identification. We obtained the information about morphological identification characteristics of exotic nematode species currently detected in plant quarantine inspection. Since 2006, nematode detection from imported agricultural crops and exotic plants was average 302 cases in every year. Newly identified species were 8 species belonging to 7 genus, 6 families including Anguina sp., Aphelenchoides haguei, Ditylenchus khani, Meloidogyne chitwoodi, Meloidogyne sp., Paraphelenchus acontioides, Rotylenchulus reniformids, and Rotylenchus arsenjevi. Both Anguina sp. and Meloidogyne sp. were unable to diagnose the exact species due to the deficit of the nematode number detected from the sample and the sources of relative literature. Both species were designated as the unrecorded species in Korea. We provided images, measured value and morphological characters of newly identified species in quarantine inspection.

Keywords:*Nematode, Plant quarantine, Korea*

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PS1M070

Taxonomy

P1

A new species and two new records of the family Neanuridae from Korea

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The family Neanuridae belongs to order Poduromorpha in class Collembola and approximately 1404 species in 171 genera have been known worldwide. It is over 6.37% of all described species of Collembola. However, only 75 species in 24 genera have been recorded from Korean peninsula. A new species of Pseudachorutes and two unrecorded species, Morulina pawlowskii, Lobella sokamensis, are recognized in Korea. The new species differs in the body chaetotaxy, the number of lobes of PAO, and the shape of mandible compare to relative species. Herein, a brief descriptions and diagnostic photographs/or figures of three Korean Neanurid species are presented with a taxonomic key of their genera for identification. The comparison of differentiating characters of all Pseudachorutes species from South Korea are given as well.

Keywords:*Collembola, Pseudachorutes, Morulina pawlowskii, Lobella sokamensis, chaetotaxy.*

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PS1M071

Taxonomy

P1

DNA barcode of arthropod pests from pine mushroom from Korea and imported

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This study was carried out for pest risk analysis of pine mushroom imported from China and North Korea, compariing pests from imported pine mushroom with from Korean pine mushroom. Pests of pine mushroom were usually found as immatual stage, so needed to rear them to adults. It was hard to identify larvae into species level, DNA BArcode (COI region of Mitochondria) from each species sample were compared, to confirm if they were same speceis or not. The number of arthropod species from pine mushroom in Korea was 149 samples, under 15 families 7 orders. The pests collected on surface of mushroom were 6 sepcies 6 families in Coleoptera, 2 species 1 family, Hymenoptera, 2 species 2 families, Diptera, 1 Thysanoptera, several Colembola, a mite, and a milipoda. The number of family of pest attacking on inner side of mushroom was 6 families of Diptera, according to DNA barcode analysis, 2 sepceis in Sciaridae, 10 in Drosophilidae, 4 Phoridae, 1 Mycetophilidae, 1 Tipulidae, and 1 Psychodidae were found. On the other hand, DNA barcodes of 4 species (2 in Phoridae, and 2 Drosophilidae) were acquired after analysis of seuencing of several fly samples inspected in pine mushroom from China. As a result of comparison of DNA barcodes, one species of Drosophilidae was coincide with Korean species, but the others were different from Korean.

Keywords:*pine mushroom, pest insect, DNA barcode, Korea, China*

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PS1M072

Taxonomy

P1

Morphometrics of the final instar exuviae for four cicada species occurring in urban areas of central Korea

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Cicadas that produce loud calling songs for mate attraction can be a nuisance to city dwellers in Korea. The exuviae of final instars can be used for estimation of population density. To provide a key for species identification, we investigated morphological characteristics of final instar exuviae for four cicada species that are abundant in urban areas of central Korea: Cryptotympana atrata, Meimuna opalifera, Oncotympana fuscata, and Graptopsaltria nigrofuscata. The characters analyzed were pro-mesonotum length, pronotum width, pronotum length, head width, mesonotum length, body length, wing length, and femoral tooth angle. The results of a general linear model showed that species and sex were significant for morphological characters, and the result of post hoc analyses revealed that the mean values of most of the morphological characters measured were significantly different among four cicada species. C. atrata and M. opalifera were separated from the other species in most characters measured. Although O. fuscata and G. nigrofuscata were not distinguishable based on size-related characters, these two species differed in femoral tooth angle. Therefore, the exuviae of all four cicada species can be easily distinguished based on the morphological characters used in this study.

Keywords:*Cryptotympana atrata, exuvia, Graptopsaltria nigrofuscata, Meimuna opalifera, Oncotympana fuscata*

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PS1M073

Taxonomy

P1

A new species of the genus *Adoxophyes* (Lepidoptera: Tortricidae) on the sweet cherry imported from China under the plant quarantine in Korea

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A new species, Adoxophyes cerasus Park, is described, illustrated, and distinguished from closely resembling species. Larvae of this species were discovered on sweet cherry (Prunus avium) imported from China and intercepted under plant quarantine inspection at Korean sea- and airport in 2008. They were reared to adult in the laboratory for identification. We also collected a male specimen from China for this study. We provide a full description of the new species based on adult of external structures. In addition, we conducted a comparative study of three related species (A. orana, A. hanmai, and A. paraorana) and provided a key to distinguish them. To confirm the identity of new species, we compared DNA barcoding sequences (658 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) gene from 13 specimens of four Adoxophyes species. As a result of this study, we confirmed the identity of Adoxophyes new species. We also provided pairwise p-distances among the species and their neighbor-joining tree.

Keywords:*Tortricidae, Adoxophyes, A. cerasus, New species, DNA barcode, China, Korea*

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PS1M074

Taxonomy

P1

Xyleborini Ambrosia Beetles of Melanesia: Taxonomic revision and monograph

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We revised the genera and species of Xyleborini (Coleoptera, Curculionidae: Scolytinae) recorded from New Guinea and neighboring islands. We examined over 44,000 representatives of 281 species, including 264 type specimens. The classification used here was based on the Catalog of Scolytidae and Platypodidae (Wood & Bright, 1992, Bright & Skidmore, 1997, 2002), and several recent cladistic and molecular analyses of Xyleborini. Morphological characters inferred by these analyses as phylogenetically informative were used to define genera and species. Many previously described species were found to be delimited by uninformative characters, 59 such species are synonymized. Three new genera are defined (Ancipitis, Immanus, Stictodex), and 20 new combinations are given. The monograph is complemented by photographs of all species and multiple-choice electronic keys to all species and genera, both available on-line. Because all the genera and many species occurring in New Guinea also occur throughout the Eastern Paleotropic region, the presented re-classification is significant for Xyleborini taxonomy on much larger geographical scale, in many cases for world-wide classification.

Keywords:*ambrosia*

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PS1M075

Taxonomy

P1

Open the gates to the diversity of Protura

Carol Resch[1], Gerald Timelthaler[2], Nikolaus Urban Szucsich[3], Daniela Bartel[4], Guenther Pass[5]

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Protura are small entognathous hexapods living in the soil. The hidden life style is but one reason why this group has proven difficult to be studied. Challenges start at the very beginning of every scientific approach. Due to their small size and due to the lack of conspicuous characters, species determination is a rare skill, restricted to a handful of scientists. The minuteness of Protura, along with the difficulties to determine specimens to species level makes sampling a hard task. Then again Protura are among the oldest lineages of hexapods, and thus indispensable to understand the evolution of the most diverse animal group on earth. Aim of our study was to address proturan diversity by sequencing the barcoding region of the CO I gene and a fragment of the 28S rRNA. The study was triggered by the close vicinity of our university to the biodiversity hotspot of Protura. In 2004 Christian and Szeptycki mentioned 23 proturan species to occur syntopically in the outskirts of the city of Vienna. Standard protocols and primers had to be modified in order to be applicable for proturans. We used a non-destructive method of DNA extraction, which leaves the cuticle intact for further morphological analyses (e.g. CLSM). Methods will be presented, along with first results on the molecular diversity of Austrian Protura.

Keywords:*Hexapoda, Protura, DNA barcoding, Austria*

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PS1M076

Taxonomy

P1

Real-time tracing of AcMNPV infection to *Plutella xylostella* and the interaction between virus and parasitoid

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Baculovirus is an important microorganism for controlling insect pests in the field, and is also crucial for integrating into an ecologically sound IPM program. In order to trace baculovirus infection, a recombinant baculovirus, vAcGFP, derived from Autographa californica nucleopolyhedrovirus (AcMNPV) was constructed. This virus contains a wt polyhedrin gene and a GFP coding region driven by p10 promoter. The LC50 values of vAcGFP and wt AcMNPV to the 3rd instar larvae with the diet incorporation method were 34.4 and 25.6 OBs/mm3 of diet, respectively. The LT50 values of these two viruses were relatively close. The larvae infected with vAcGFP were easily detectable due to the emittion of green fluorescence (505nm) as irradiated by a 365nm UV light. Its FT50 (50% fluorescense-emitting time) was 65 h earlier than LT50 at 763 OBs/mm3. The 3rd instar larvae of P. xylostella were inoculated with vAcGFP and simultaneously parasitized by the parasitoid, Cotesia plutellae. A fluorescence-emitting rate of 84 and 32% was separately coupled with 12 and 54% cocooning rate at 76.3 and 7.6 PIBs/mm3, respectively, and only 5~8% cocoons with fluorescence was detected. The cocooning rate of C. plutellae was decreasing through the time of parasitism and the increasing of viral concentration. In conclusion, GFP could be used as a specific and non-destroying biomarker for detection of insect dynamics after being infected with NPV in the field. In addition, our study shed a light for further study of the complicated interactions between baculovirus and broconid wasp in an insect host.

Keywords:*Autographa californica nucleopolyhedrovirus (AcMNPV), Green fluorescence protein (GFP), recombinamt virus, Plutella xylostella, Cotesia plutellae*

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PS1M077

Taxonomy

P1

New record for the Turkish house fly (Muscidae: Diptera) fauna

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The Muscidae one of the largest family of the calyptrate Diptera that contains almost 4500 described species over 86 genera. The world-widespread distributed Muscidae is presented within approximatelly 620 species in Europe and it constitutes a large portion of Diptera fauna on the country. This work provides the first country record for the species extending the known range of the species and taxonomic characters were given in details.As a result of many researches, the Muscidae, forensically third important family is dedicated as significant on forensic cases. In this study, specimens of Mesembrina meridiana (Linnaeus 1758) which is a new record for the Turkish fauna, were collected on different days of pig decomposition during a forensic entomology research, in Eskisehir. The specimens were collected on 3th and 8th days of decomposition, fresh and bloat stages when Calliphoridae and Sarcophagidae species not certainly active due to seasonal activity periods. The preferred habitat of this species, ovulation activity zone preference, and availability for forensic investigations briefly discussed in this study.

Keywords:*Muscidae, New Record, Mesembrina meridiana, Forensic Entomology, Diptera, Turkey*

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Taxonomy

P1

Determination of the phylogeny of some tabanidae (Diptera : Tabanidae) species using coi gene area

Ali Yavuz Kilic[1], Sukran Yalcin[2]

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The Tabanidae Bromius group (Insecta: Diptera) members had been tried to identfy from their universal gene areas. Specimens were collected from different areas of Turkey Collected specimens' cytochrome oxidase one gene area were amplified with one of the universal primer COI, through polymerase chain reaction and had done sequencing. Achieved sequences analysed with MEGA5 program, BOLDsystems, Kimura 2 parameter and General Time Reversible Gamma disribution model comparing genebank sequences, than had got phylogenetic trees using Neighbor joining and Maximum Likelihood statistical methods. Based on the results obtained from phylogenetic trees the group members are geographically divided into 7-8 groups in their species group which proved their different populations. According to analysis results that are difficult to distinguish morphologically from each other, T. maculicornis, T. miki and T. armeniacus bootstrap value of 90% shared same klad on the trees. Nearest-Neighbor-Interchange method gives optimal trees of the results. According to the these optimal trees Neighbor joining and Maximum likelihood trees figure in attachment-4 specimens' sequences results are divided into two groups and the trees do not include any gene bank specimen. In fact, there are not any documented data about Bromius group specimen from Turkey at the gene bank, so comparing is not possible. Because of that, this study is very important including first data about bromius genes from Turkey. According to numerous analysis it is obvious that difficult to distinguish morphologically from each other, T. maculicornis, T. miki and T. armeniacus shared same klad a very high bootstrap value of 90%.

Keywords:*Phylogenetic Relationships, Tabanus bromius, Tabanidae, Diptera*

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Taxonomy

P1

Occurrence of grape rust mite, *Calepitrimerus vitis*, in Korea

Myoung Rae Cho[1], Jong Ho Lee[2], Taek Jun Kang[3], Hyung Hwan Kim[4], Seung Joon Ahn[5], Seung Wook Jeon[6], Hae Woong Park[7]

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Grape rust mite, Calepitrimerus vitis, is one of the major pests in the vineyards in the world. The mite was recently found in grape farms and the taxonomic characteristics and the distribution are reprted for the first time in Korea. The female morpholgy are : Body fusiform, 170-190 long, 50-52 wide. Gnathosoma directed down, 20-25 long. Prodorsal shield broadly triangular, 33-36 long, 38-43 wide; frontal lobe overhanging gnathosoma, bilobed; prodorsal shield with median and admedian lines and granular lateral lines; dorsal tubercles ahead of rear margin; setae directed up and centrad. Dorsal annuli 67-72, slightly less than ventral annuli, 70-74, terminal annuli 5; three-fourths of dorsal annuli with middorsal ridge; dorsal annuli lobed dorsally and with faint, elongate, rounded microtubercles touching rear annular margins laterally. Ventral annuli with small rounded microtubercles touching rear annular margins. Setae h1 present, 5 long. Coverflap with 2-3 transverse granular lines basally, 10-12 longitudinal ridges, setae 13-14 long. Coxae with granulation, sternal line simple and faint. Empodia 5-rayed; tarsal solenidia knobbed. The damage symptom in greenhouse grape was delayed growth of new leaves and flowers. A survey on some major grape production area revealed that the mite is distributed in Hwasung, Ansung, Gapyung, Chunan, Okchun, Jeonju, Kimje and Kimchun. In the twig sample collected from the areas, the mite was found in the surface and ovewintering buds.

Keywords:*Grape rust mite, Calepitrimerus vitis, taxonomy, overwinter*

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PS1M080

Taxonomy

P1

Taxonomic review of the genus *Zeiraphera* (Lepidoptera, Tortricidae) in Korea

Bong-Kyu Byun[1], Sat-Byul Shin[2]

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The genus Zeiraphera belongs to the tribe Eucosmini belonging to the family Tortricidae, comprising more than 1,600 described species worldwidely (Brown, 2005). In Korea, totally six species have been listed by Byun et al (2009). Recently Byun (2011) added a species Zeiraphera lariciana Kawabe, 1980 from North Korea. In the present study, we review the genus Zeiraphera in the Korean peninsula. Also, the North Korean fauna for the genus is noted, based on the material of Hungarian Natural History Museum, Budapest, Hungary. In this study, a total of seven species is recognized from Korea with description of a new species and new faunistic data. All the known species is enumerated with illustration of their adult, male and female genitalia. A taxonomic list of the genus is as follows. Genus Zeiraphera Treitschke, 1829 1. Zeiraphera demutana (Walsingham, 1900) 2. Zeiraphera fulvomixtana Kawabe, 1974 3. Zeiraphera griseana (Hubner), 1799 4. Zeiraphera rufimitrana (Herrich-Schaffer, 1847) 5. Zeiraphera virinea Falkovitsh, 1965 6. Zeiraphera lariciana Kawabe, 1980 7. Zeiraphera paravirinea sp. nov.

Keywords:*Lepidoptera, Tortricidae, Eucosmini, Zeiraphera, Taxonomy, Korea*

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PS1M081

Taxonomy

P1

A preliminary checklist of the family Psychidae(Lepidoptera) in Korea

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The family Psychidae, so- called bagworms, of Korea is reviewed. A total of 1324 species of the family Psychidae have been described in the world to date (Sobczyk, 2002). However, the Psychidae of Korea has been poorly studied to date, including only 7 recorded species (Park et al., 1983; Byun et al., 2009.). In the present study, we reviewed and made a preliminary list of Korean Psychidae with their taxonomic illustration and available information, including locality, host plant and biological characteristics. Family Psychidae Boisduval, 1828 1.Mahasena aurea (Butler), 1881, Trans. Ent. Soc. London 1991: 23. 2.Eumeta japonica Heylerts, 1884, Annl. Soc. Ent. Belg. 28, Compt. Rend.: 11. 3.Eumeta minuscula Butler, 1881, Trans. Ent. Soc. London, 1881: 22. 4.Acanthopsyche nigraplaga Wileman, 1911, Trans. Ent. Soc. Lond. 1881: 347. 5.Kozhantshikovia vernalis Saigusa, 1961, Sieboldiia 2: 305. 6.Taleporia nigropterella Saigusa, 1961, Sieboldia 2: 297. 7.Taleporia trichopterella Saigusa, 1961, Sieboldia 2: 292

Keywords:*Lepidoptera, Psychidae, bagworms, taxonomy, Korea*

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PS1M082

Taxonomy

P1

Historical review of Gracillariidae (Lepidoptera) in Korea

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The family Gracillariidae has been known as a typical leaf-miners, with more than 2,300 described species in the world (Prins & Prins, 2005). In Korea, the first report on the familiy was made by Kumata et Park (1978) with description of a new species of the genus Phyllonorycter Hubner from Korea. Later thirteen species of three genera of the subfamily Lithocolletinea are reported from Korea (Kumata et al 1983). Park (1983) provided the checklist on this group of Korea with 26 species. Then, Park and Han (1986) reported six newly reported species with a list of thirty-six species of Gracillariidae in Korea. Park (1994) listed 38 species of the family. Recently forty-five species were enumerated in "A Checklist of the Microlepidoptera in Korea" (Byun et al., 2009). This study is aimed to review and clarify the fauna of the family Gracillariidae with available information. In the present study, a total of 45 species of 12 genera under two subfamilies are listed with taxonomic information including their collecting locality, host plant, and distributional range. Also a taxonomic key is provided.

Keywords:*Lepidoptera, Gracillariidae, Gracillariinea, Lithocolletinea, Taxonomic, Korea*

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PS1M083

Taxonomy

P1

Efficacy of DNA barcodes to identify insect foreign material in foods: A case study using tenebrionids (Coleoptera: Tenebrionidae)

Seo Young Cho[1], Boo Hee Jung[2], Kyong In Suh[3], Yeon Jae Bae[4]

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Insect pests that are found in food are of great economic and hygienic significance. However, the precise identification of each of these species often requires an expert taxonomist, and the process can be time-consuming. Furthermore, these pests are usually discovered in food in various conditions such as in terms of body parts or immature specimens; this further complicates the identification process. For these reasons, an efficient means of identification is necessary for developing a control scheme for insect pests. In this study, we tested the efficacy of DNA barcodes to identify tenebrionid beetles and other significant food pest species. Species-level identification based on external morphology can be difficult because these pests are extremely variable in body-size and form, as well as in terms of genetic polymorphisms. Furthermore, larvae of this species have also remained uncharacterized. The 640-bp cytochrome c oxidase subunit I (COI) gene in the tenebrionid mitochondrial DNA was sequenced and analyzed for variations in inter- and intra-species COI sequence. The effectiveness of the COI?based DNA barcodes in discriminating Korean tenebrionids through integrative re-examinations with over 20 unidentified species was also examined. Pairwise sequence divergence was calculated using the Kimura two-parameter model, and a neighbor-joining tree based on calculated generic differences among and within species was constructed.

Keywords:*Food insect pests, Food foreign material, DNA barcoding, COI gene, Tenebrionidae*

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Taxonomy

P1

Development of species-specific primers for the molecular diagnosis of quarantine species in the genus *Tetranychus* and *Amphitetranychus* (Acari: Tetranychidae)

Tin Moe Khaing[1], Jae Kyeong Shim[2], Jong Ho Lee[3], Won Goo Lee[4], Kyeong Yeoll Lee[5]

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Spider mites are one of the most important mite pests of agriculture crops and also important in plant quarantine service. Morphological identification of mites can be difficult due to their small size and a few characters available and also lack of noticeable characters for immature mites. To overcome these difficulties, morphologically identified mites were compared with those of DNA sequences using internal transcribed spacer 2 (ITS2). Species-specific primers were designed according to the ITS2 sequences of 6 species in the genus Tetranychus (T. urticae, T. kanzawai, T. phaselus, T. truncatus,) and Amphitetranychus (A. quercivorus, A. viennensis) which are important in plant quarantine. Our results provide information for the accurate diagnosis of mite identification in plant quarantine.

Keywords:*Amphitetranychus, ITS2, Molecular diagnosis, Morphological identification, Spider mites, Tetranychus*

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PS1M085

Taxonomy

P1

Identification of biotypes and secondary endosymbionts of *Bemisia tabaci* in Korea and relationships with the occurrence of TYLCV disease

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Bemisia tabaci is a species complex that consists of at least 24 genetically diverse biotypes. Here, we determined the biotypes of 27 populations collected in 17 different regions of Korea. Nucleotide sequence comparisons of cytochrome oxidase showed that 26 populations were Q biotype and that one population, the Goyang population, was B biotype. Further subgroup analysis of the Q biotype showed that all populations belonged to the Q1 subgroup, which originates from Western Mediterranean countries. Five endosymbiotic bacteria from various B. tabaci populations were analysed by comparing rDNA sequences. Hamiltonella was detected in all the populations tested regardless of biotype. Cardinium was detected in all Q biotype populations but not in the B biotype population, while Rickettsia was detected in the B biotype population but not in Q biotype populations. Arsenophonus and Wolbachia were detected in 35% and 58% of Q biotype populations, respectively, but not in the B biotype population. Our results show that the endosymbiont profile is strongly associated with each biotype and with subgroups of the Q biotype. Survey of TYLCV disease from 2008 to 2010 indicated that this disease is widely spread in Korea. This study suggests that the rapid spread of TYLCV may be associated with endosymbiont infection, particularly Hamiltonella infection of B. tabaci.

Keywords:*Bemisia tabaci, Biotype, Endosymbiotic bacteria, TYLCV, Vector insects*

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PS1M086

Taxonomy

P1

Molecular identification of spiraling whitefly *Aleurodicus dispersus* (Hemiptera: Aleyrodidae) and prevalence of secondary endosymbionts

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Spiraling whitefly, Aleurodicus dispersus, is a serious pest in tropical and neotropical regions especially for south-east Asia and also important in plant quarantine. In Bangladesh, Myanmar and Philippines its damage has been devastated in fruits and ornamental plants particularly for guava. Here, we conducted molecular identification of the spiraling whiteflies collected from Bangladesh, Myanmar and Philippines. Nucleotide sequence comparisons of both mitochondrial cytochrome oxidase I (mtCOI) and 16S ribosomal RNA (16S rRNA) showed that mtCOI and 16S rRNA was most simliar to Aleurodicus dugesii and A. dugesii at rates of 90% and 91%, respectively. We designed a mtCOI specific primer set of A. dispersus for the species diagnosis of in plant quarantine. In addition, we conducted the identification of secondary endosymbionts from different populations of A. dispersus by the PCR analysis using primer sets of 16-23S rDNA sequences that can be used for the endosymbiont identification of Bemisia tabaci. Wolbachia was detected in all the tested populations. Cardinium was detected in populations of Myanmar and Philippines but not in that of Bangladesh. However, spiraling whiteflies were never be infected with Arsenophonus, Hamiltonella and Rickettsia. Both Wolbachia and Cardinium of A. dispersus was highly similar to those of Wolbachia (Cinara cedri at rates of 99% in Myanmar, Diaphorina citri at 100% in Bangladesh, and Naupactus cervinus at 99% in Philippines) and Cardinium (Encarsia berlesei at rates of 99% in Philippines and Bemisia tabaci at 92% in Myanmar) respectively. Our results show that the endosymbiont profile is strongly associated with the species

Keywords:*Aleurodicus dispersus, Cardinium, mtCOI, Spiraling whitefly, Wolbachia*

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PS1M087

Taxonomy

P1

Phylogenetic relationships of the insect order Odonata using COI, 16S rRNA, 28S rRNA and EF1-α Sequences

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A diverse phylogenetic relationship has been proposed at several taxonomic levels for the insect order Odonata both by morphological and molecular data. This includes the non monophyleties of each suborder Zygoptera and Anisoptera, and variable relationships among superfamilies and families. In this study, approximately 3,800 nucleotide sequences from 2 mitochondrial genes (cytochrome oxidase subunit I, COI and 16S ribosomal RNA, 16S rRNA) and 2 nuclear genes (28S rRNA and elongation factor-1 alpha, EF-1α) were sequenced from 70 species of Odonata occurring in Korean peninsula (9 families, 6 superfamilies, and 2 suborders), along with 4 outgroup species (each one species from Hemiptera and Orthoptera, and two from Ephemeroptera). These sequences were subjected to phylogenetic reconstruction via Bayesian Inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP) algorithms. In all analysis, each families and superfamilies were all consistently clustered together as monophyletic groups with a high nodal support, whereas majority of analyses placed the Lestidae belonging to Zygoptera as the most basal lineage of Anisoptera, suggesting the possibility of non monophyly of the suborder Zygoptera. Topological test to find out better supported tree further strongly supported non monophyly of Zygoptera, providing higher statistical estimates. Further scrutinized analyses will be performed to obtain further robust phylogenetic relationships.

Keywords:*Odonata phylogeny*

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PS1M088

Taxonomy

P1

Taxonomic revision of the genus *Chionea* Dalman (Diptera: Limoniidae) in Korea

Yong Jung Kwon[1], Sang Jae Suh[2]

[1]*Kyungpook National University, Republic of Korea,* [2]*Kyungpook National University, Republic of Korea*

Snow crane flies of the genus Chionea are characterized by the complete reduction of their wings and by the winter activity of adults. Although they are distributed over the Holarctic region, so far only seven species are known to come from Far East Asia: Chonea araneoides Dalman, C. kanenoi Sasakawa, C. crassipes crassipes Boheman, C. crassipes magadanensis Narchuk, C. nipponica Alexander, C. pusilla Savchenko and C. mirabilis Vanin. As far as we know, the first record of the genus from Korea was made by Savchenko (1992), who reported Chionea crassipes gracilistyla (= C. crassipes) from North Korea. Thereafter, Vanin (2008) described a new species, C. mirabilis, from Mt. Deogyusan (500-600m), in the southern region of the South Korea with the record of C. crassipes. Therefore, only two species have been known to Korean fauna. In this paper, a total of three Korean species of the genus Chionea are treated. Among them, C. kanenoi Sasakawa, 1986 is recorded for the first time in Korea.

Keywords:*Taxonomy, Chionea, Korea*

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Taxonomy

P1

A revision of the Aulacidae (Hymenoptera: Evanioidea) with two new species from Korea

Jin-Kyung Choi[1], Jong-Wook Lee[2]

[1]National Institute of Environmental Research, Republic of Korea, [2]Yeungnam University, Republic of Korea

The family Aulacidae currently includes 240 extant species placed in three genera: Aulacus Jurine, 1807 (76 species), Pristaulacus Kieffer, 1900 (164 species), and Panaulix Benoit, 1984 (2 species). Members of this family are distribute in all zoogeographic regions except Antarctica (Kieffer 1912; Hedicke 1939; Benoit 1984; Smith 2001, 2005a,b, 2008; He et al. 2002; Jennings et al. 2004a,b,c; Turrii 2004, 2005, 2006, 2007, 2011a,b; Jennings & Austin 2006; Sun & Sheng 2007a,b; Lee & Turrisi 2008). Recent reports (Konishi 1990; Mason 1993; Gauld 1995) recognized Aulacidae as a distinct family among the Evanioidea, and not a subfamily of the Gasteruptiidae, as reported by some authors (e.g. Rasnitsyn 1988). Also, Jennings and Austin (2000) and Turrisi (2004) provided evidence for a monophyly of the Aulacidae, based upon cladistic analysis. This family is parasitoids of wood-boring Hymenoptera and Coleoptera, and they use a koinobiont endophagous strategy (Skinner & Thompson 1960; Whitfield 1998; Jennings & Austin 2004). In this study, we report the family Aulacidae with two new species, Pristaulacus n. sp. candidate 1 and Pristaulacus n. sp. candidate 2. Including the two new species, the number of Korean Aulacidae is now seven: Aulacus salicius Sun and Sheng, 2007, Pristaulacus insularis Konishi, 1990, P. intermedius Uchida, 1932, P. kostylevi Alekseev, 1986, P. jirisani Smith and Tripotin, 2011, P. n. sp. candidate 1, and P. n. sp. candidate 2. A detailed descriptions, figures of morphological features, and a key to the Korean Aulacidae are provided.

Keywords:*Aulacidae, New Species, Korea*

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Taxonomy

P1

Descripion of immature stages of *Tipula(Nippotipula) coquilletti* (Insecta, Diptera, Tipulidae) from Korea with notes its ecology

Jaeick Jo[1], Dongsoo Kong[2]

[1]Kyonggi University, Republic of Korea, [2]Kyonggi University, Republic of Korea

The external anatomy of the last instar larva and pupa of the crane fly, Tipula(Nippotipula) coquilletti Enderlein, 1912 (Diptera: Tipulidae), is described and illustrated from specimens collected in Korea. Comments concerning natural history and microhabitats of larvae are provided. This is the first detailed description with illustrations for the larva and pupa of Tipula(Nippotipula) coquilletti. Pupal characters resemble those of most species from advanced lineages within the subfamily Tipulinae.

Keywords:*Diptera, Tipulidae, Immature stages, South Korea*

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Taxonomy

P1

Molecular phylogeny and estimates of divergence times of Nymphalidae (Lepidoptera: Papilionoidea) based on concatenated mitochondrial and nuclear genes

Xinlong Wan[1], Min Jee Kim[2], Iksoo Kim[3]

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The phylogenetic relationships among the Nymphalidae (Lepidoptera: Papilionoidea) have been controversial in several perspective. The present study sequenced a total of ~ 3,404 bp from cytochrome oxidase subunit I (COI), 16S ribosomal RNA (16S rRNA), and elongation factor-1 alpha (EF-1α) in 80 nymphalid species belonging to seven subfamilies (Linmenitidinae, Heliconiinae, Nymphalinae, Apaturinae, Libytheinae, Satyrinae, and Danainae), along with those of six species of Pieridae as outgroups. Phylogenetic analyses consistently supported the subfamilial relationships of (((((Linmenitidinae + Heliconiinae) + (Nymphalinae + Apaturinae)) + Satyrinae) + Libytheinae) + Danainae), with a high nodal support for monophyletic subfamilies and tribes. This result is largely consistent with a previous study performed with substantially large sequence information and morphological characters, but the positions of Libytheinae and Danainae are exchanged. For the relationships among genera within subgroups of Nymphalidae, Coenonympha was consistently placed as the basal lineage of the Satyrii with strong supports. In tribe Argynnini, the genus Argynnis was supported monophyletic and sister to Brenthis. The genus Aldania always was included in the group of Neptis within tribe Neptini, resulting in Neptis non-monophyletic, which is probably related to the origin of Aldania. The analysis of divergence times among subfamilies suggests that Nymphalidae began diversifying at around 96 million years ago (MYA), with Danainae branching off the common ancestor of the rest of Nymphalidae. The remaining subfamilies diverged between 62.16 MYA and 89.56 MYA, suggesting that all subfamilies in Nymphalidae have diverged in late Cretaceous or within short time after Tertiary.

Keywords:*Phylogenetic relationship, mitochondrial COI and 16S rRNA, nuclear EF-1α, divergence time, and Nymphalidae*

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Taxonomy

P1

Complete mitochondrial genome of the free-living earwig, *Challia fletcheri* (Dermaptera: Pygidicranidae) and phylogeny of Polyneoptera

Xinlong Wan[1], Min Jee Kim[2], Iksoo Kim[3]

[1]Chonnam National University, Republic of Korea, [2]Chonnam National University, Republic of Korea, [3]Chonnam National University, Republic of Korea

The insect order Dermaptera, belonging to Polyneoptera, includes ~2,000 extant species, but no dermapteran mitochondrial genome has been sequenced. We sequenced the complete mitochondrial genome of the free-living earwig, Challia fletcheri, compared its genomic features to other available mitochondrial sequences from polyneopterous insects. In addition, the Dermaptera, together with the other known polyneopteran mitochondrial genome sequences (protein coding, ribosomal RNA, and transfer RNA genes), were employed to understand the phylogeny of Polyneoptera, one of the least resolved insect phylogenies, with emphasis on the placement of Dermaptera. The complete mitochondrial genome of C. fletcheri presents the following several unusual features: the longest size in insects is 20,456 bp; it harbors the largest tandem repeat units (TRU) among insects; it displays T- and G-skewness on the major strand and A- and C-skewness on the minor strand, which is a reversal of the general pattern found in most insect mitochondrial genomes, and it possesses a unique gene arrangement characterized by a series of gene translocations and/or inversions. The reversal pattern of skewness is explained in terms of inversion of replication origin. The markedly different gene arrangement of C. fletcheri is hypothesized to be due to some unique organism-level properties which allow relaxed selection against mitochondrial gene rearrangement. All phylogenetic analyses consistently placed Dermaptera as the sister to Plecoptera, leaving them as the most basal lineage of Polyneoptera or sister to Ephemeroptera, and placed Odonata consistently as the most basal lineage of the Pterygota.

Keywords:*Mitochondrial genome, Dermaptera, and Phylogeny of Polyneoptera*

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Taxonomy

P1

A taxonomic study on the Genus *Linnaemya* Robineau-Desvoidy (Diptera, Tachinidae) in South Korea

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Robineau-Desvoidy (1830) erected the genus Linnaemya based on the type-species, L. silvestris Robineau-Desvoidy. The world fauna of the genus currently includes 149 nominal species, including 51 Palaearctic, 42 Oriental, seven Nearctic, five Australian, and 64 Afrotropical species. Only three species were known from South Korea up to date. They are charaterized as follows: 1) parafacials bare with normally three sternopleural setae, 1+3 intra-alar setae; 2) epistome prominent but not strikingly nasute; 3) vibrissae inserted above the level of the epistomal margin by a distance less than the length of the second antennal segment; and 4) posternal membrane and prosternum bare. As the result of the present study, I recognize one possible new species and seven new records have been recognized from South Korea. In addition, morphological phylogenetic analyses were conducted to infer interspecific relationships among Linnaemya species using species of the genus Lypha as an outgroup.

Keywords:*Taxonomy, Diptera, Tachinidae, Linnaemya, South Korea*

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Taxonomy

P1

Insect expedition of vitnam (Butterflies)

Eun Young Choi[1], Jinyoung Park[2], Ik Je Choi[3], Yong Kyun Oh[4], Kyu Tek Park[5], Yang Seop Bae[6], Pham Thi Vuong[7], Jong Kyun Park[8]

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Vietnam is located on the eastern Indochina Peninsula between the latitudes 8° and 24° N, and the longitudes 102° and 110° E. It covers a total area of approximately 331,210 square kilometers (127,881 sq mi), excluding the Hoang Sa and Truong Sa islands, making it almost the size of Germany. The combined length of the country's land boundaries is 4,639 kilometers (2,883 mi). During the winter or dry season, extending roughly from November to April, the monsoon winds usually blow from the northeast along the China coast and across the Gulf of Tonkin, picking up considerable moisture. Temperatures vary less in the southern plains around Ho Chi Minh City and the Mekong Delta, ranging between 21 and 28 °C (69.8 and 82.4 °F) over the course of a year. Seasonal variations in the mountains and plateaus and in the north are much more dramatic, with temperatures varying from 5 °C (41 °F) in December and January to 37 °C (98.6 °F) in July and August. This expedition was conducted two times in two years (2003, 2004). Sweeping method, light trap and searching etc., were used to collect insect. Up to now, 35 species of 4 families(Nymphalidae, Pieridae, Papilionidae, Lycaenidae) were confirmed. Collected species, collecting sites, collecting method, collected specimens, ecological notes and activities of Vietnam will present with photos.

Keywords:*Taxonomy, Lepidoptera, Butterflies, Vietnam*

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Taxonomy

P1

Insect expedition of Vietnam (Beetles)

Ik Je Choi[1], Jinyoung Park[2], Eun Young Choi[3], Yong Kyun Oh[4], Kyu Tek Park[5], Yang Seop Bae[6], Pham Thi Vuong[7], Jong Kyun Park[8]

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Vietnam is located on the eastern Indochina Peninsula between the latitudes 8 ° and 24 °N, and the longitudes 102 ° and 110 °E. It covers a total area of approximately 331,210 square kilometers (127,881 sq mi), excluding the Hoang Sa and Truong Sa islands, making it almost the size of Germany. The combined length of the country's land boundaries is 4,639 kilometers (2,883 mi). During the winter or dry season, extending roughly from November to April, the monsoon winds usually blow from the northeast along the China coast and across the Gulf of Tonkin, picking up considerable moisture. Temperatures vary less in the southern plains around Ho Chi Minh City and the Mekong Delta, ranging between 21 and 28 °C (69.8 and 82.4 °F) over the course of a year. Seasonal variations in the mountains and plateaus and in the north are much more dramatic, with temperatures varying from 5 °C (41 °F) in December and January to 37 °C (98.6 °F) in July and August. This expedition was conducted two times in two years (2003, 2004). Pitfall trap, sweeping method, light trap and searching etc. were used to collect insect. As the result, totally 10 families in Coleoptera, mainly species in Scarabaeidae, Lucanidae, Meloidae and Cerambycidae etc., were collected. Among Coleoptera species, with big sized beetles photos for species of dominant, and also collecting sites, collecting method, special character of vietnam insect and activities were presented.

Keywords:*Taxonomy, Coleoptera, Beetles, Vietnam*

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Taxonomy

P1

The insect fauna of Dokdo

Yong Kyun Oh[1], Jinyoung Park[2], Ik Je Choi[3], Eun Young Choi[4], Jong Kyun Park[5]

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Dokdo is an island situated in the east longitude 131° 52′ and the north latitude 37° 14′. The full address for Dokdo is: 1-37 mountains, Dokdo-ri, Uleung-eup, Uleung-gun, Gyeongsangbuk-do, South Korea also the islets are located in the sea area 90 km southeast to Uleungdo and east of the Korean Peninsula. Dokdo is consisted of 2 main islands called Dongdo and Seodo and 33 attached small rocks and reefs. The gross area is 186,121㎡. Dokdo is important to migratory bird because it is on the migratory bird's flyway. Insect Fauna of Dokdo is researched intermittently and incompletely. However, it is very significant geographical, economical and militarily. The important value was recognized culturally and it have been many investigations later 2000. As a result 132 species belonging to 129 genera of 65 families in 11 orders recorded in Dokdo from 2010~2011 by our research. Species list photo of main specimens and historical records etc. are provided here in.

Keywords:*Dokdo, Insect fauna, Korea*

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PS1M097

Taxonomy

P1

Newly recorded family Heliozelidae from Korea

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The family Heliozelidae includes very small-sized moths with forewing length of 3-9mm, comprising 12 genera and about 100 species in the world. It has a worldwide distribution mainly in the Nearctic and Australian regions, but it has not been known from Korea. The adults of Heliozelidae are seldom collected, because of their small size and diurnal habit. In addition, this family is leaf miner through the larval stage and it has single generation for the year. Heliozelidae lacks cross-vein M-CuA on the hindwing. It is principle autapomorphic character for the family. The larvae of almost genera are known to mine in a leaf of trees and shrubs of Myrtaceae, Vitaceae, Cornaceae, Fagaceae, Saxifragaceae, etc., except only one genus, Antispilina Hering, 1941, which is known to mine in herbaceous plants. Recently, participating in a research project of Korea National Arboretum, we collected several heliozelid species, Antispila hikosana, A. purplella, Heliozela limbata and so on, in Korea. In the present study, male and female genitalia, abdomen of adults are described.

Keywords:*Heliozela, Heliozelidae, Lepidoptera, Korea*

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Taxonomy

P1

Confirmation of the fifth mitochondrial DNA lineage of the western honeybee from Sudan

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Using mtDNA variability, the Apis mellifera subspecies could be classified into five major lineages: 1) lineage A including A. m. intermissa, A. m. adansonii, A. m. scutellata, A. m. capensis and A. m. monticola subspecies; 2) lineage M composed of the western European subspecies A. m. mellifera and A. iberiensis ;3) lineage C composed of A. m. ligustica, A. m. carnica and A. m. caucasica ;4) lineage O comprising A. syriaca and A. lamarckii; 5) lineage Y with A.m jemenitica. Apis mellifera jemenitica occupied a large area extend from Mali in west Africa through Sudan to the Arabian peninsula in Asia. However its classification is still controversial in morphometrical as well as genetic studies. The Y lineage which had recently been classified in A. m. jemenitica of Ethiopia, has never been detected elsewhere even in the same subspecies from the Neighboring countries. Moreover very recent morphometrical analyses described honeybee of Ethiopia as a single new subspecies, Apis mellifera simensis. In this paper, we aim to resolve this contradiction of classifying A.m.jeminitica. Using Dra I analyses and the partial sequence of the tRNAleu COII region of 30 sampled colonies along the river Nile in Sudan, we detected three different mtDNA lineage: A,O,and Y, each lineage compose of suit of haplotyps. We found the Y-lineage in 10% of the sampled colonies. This result report the existence of Y lineage in A. m. jemenitica of Sudan for the first time and gives further confirmation to the Y lineage in central and east Africa

Keywords:*Apis mellifera jemenitica, subspcies, mitochondrial DNA, Sudan*

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Taxonomy

P1

On caligonellid mites (Acari: Trombidiformes: Prostigmata) of Iran

Mohammad Bagheri[1], Mansoureh Ahani Azad[2]

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Mites of the family Caligonellidae are relatively small, reddish-colored, free-living predatory mites that feed on small arthropods. They often live on tree bark and in litter, soil, moss, storehouses and bird's nests. To date 11 species of this family bleonging to four genera, namely Neognathus Willmann, Molothrognathus Summers & Schlinger 1955, Caligonella Berlese 1910 and coptocheles Summers and Schlinger 1955, have been recorded from Iran. In a study of caligonellid mites of Northwest Iran, we collected 10 species of this family as bellow which two species are new species for caligonellid mites of world and marked by one asterik: Neoganthus terrestris (Summers & Schlinger, 1955) ; N. ueckermanni Bagheri, Do?an & Haddad, 2010; N. eupalopus Meyer & Ueckermann, 1989; N. spectabilis (Summers & Schlinger, 1955); Molothrognathus n. sp.1*; Molothrognathus n. sp.2 * ; M. bahariensis; M.azizi; M. mehrnejadi Liang & Zhang, 1997 and Caligonella humilis (Koch, 1838).

Keywords:*Acari, Trombidiformes, Prostigmata, new species, Iran*

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PS1M100

Taxonomy

P1

Mites of family Stigmaeidae (Acari: Trombidiformes) in Bonab orchards (Northwest Iran), with one new species of the genus *Stigmaeus* Koch, 1836

Saeede Sadat Gheblealivand[1], Mohammad Bagheri[2], Hamed Ghorbani[3]

[1]*University of Maragheh, Iran,* [2]*University of Maragheh, Iran,* [3]*Dryland Agricultural Researcher Instiute, Iran*

Stigmaeidae is the second most frequent and abundant group of predatory mites on plant leaves, after Phytoseiidae which feed on a variety of arthropods. They are small, red, yellow or orange mites that occur in many habitats and form an important component of the acarofauna of soil, litter and plants. In order to study of stigmaeid mites fauna of apple orchards of Bonab (East Azerbaijan province), sampling was taken during 2010 and the mites were extracted and cleared in Nessbit fluid and microscopic slides prepared. In this study, 6 genera and 13 species identified as fallows, of which one species is new and marked by two asterisks. Cheylostigmaeus gharekhanii Navaei-Bonab and Bagheri, 2011; Eustigmaeus nasrinae Khanjani & Ueckermann, 2002; E. segnis (Koch), Wood, 1973; E. setiferus Bagheri, 2011; E. azerbaijaneansis Haddad, 2011; Ledermuelleriopsis plumosa Willmann, 1951; L. zahiri Khanjani and Ueckermann, 2002; Stigmaeus boshroyehnsis Khanjani, 2010; S. elongatus Berlese, 1886; S. shabestariensis Haddad, Lotfollahi and Akbari, 2010; Stigmaeus sp.**, Storchia robustus (Berlese), Oudemans, 1923; Zetzellia mali (Ewing), Summers, 1960.

Keywords:*Stigmaeidae, Trombidiformes, Stigmaeus, new species, Iran*

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PS1M101

Taxonomy

Morphological classification of Subfamily Dacinae (Diptera: Tephritidae) in China

Qinge Ji[1], Jiahua Chen[2], Nannan Zhang[3]

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Tephritidae (Insecta: Diptera) is one of the largest families which have economic significance. It includes 3 subfamilies i.e. Dacinae, Tephritinae and Trypetinae. More than 4500 species and 500 genera have been recorded in the world. Dacinae includes 3 tribes i.e. Ceratitidini, Gastrozonini and Dacini. Dacini includes 2 genera i.e. Bactrocera Macquart and Dacus Fabricius. Considerable concern has been expressed within Bactrocera Macquart for species complexes occurred. In this paper, more than 35000 specimens of Dacinae, collected from Henan (Luoyang), Jiangsu (Jintan, Nanjing), Chongqing, Jiangxi (Fuzhou), Fujian (Xiamen, Fuzhou, Zhangzhou, Gutian and Putian), Guangxi (Nanning, Guilin), Guangdong (Dongguan, Guangzhou), Guizhou (Guiyang), Yunnan (Jinghong, Yuanjiang and Jianshui), Hainnan (Wenchang, Danzhou and Sanya) in mainland China were identified. These specimens included 41 species described taxonomically and belonged to 2 tribes and 3 genera respectively, among them, 11 species and 2 records were new of China. The 11 new species were B. (B.) cheni, sp. nov., B. (B.) guiyangensis, sp. nov., B. (B.) hardyi, sp. nov., B. (B.) jinghongensis, sp. nov., B. (B.) nanningensis, sp. nov., B. (B.) nigrifacia, sp. nov., B. (Javadacus) ziai, sp. nov., B. (Z.) baoshanensis, sp. nov., B. (Z.) hendeli, sp. nov., B. (Z.) proprescutellata, sp. nov. and B. (Z.) zhaotongensis, sp. nov. Two new records were B. (B.) zonata (Saunders) and B. (Z.) vultus Hardy. 11 new species were described in English. Figures of 41 species and key to species were provided. All specimens are deposited in Fujian Agriculture and Forestry University.

Keywords:*Morphological classification, Bactrocera*

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PS1M102

Taxonomy

P1

On the *Anoplocheylus* Berlese, 1910 (Acari: Pseudocheylidae) mites of Iran, with description of two new species

Elham Zarei[1], Mohammad Bagheri[2], Mansoureh Ahaniazad[3]

[1]University of Maragheh, Iran, [2]University of Maragheh, Iran, [3]University of Maragheh, Iran

In order to study of Anoplocheylus Berlese,1910 species fauna of Iran, samples were taken from the soil of orchards and crop fields during 2010. By using the Berlese funnel, mites were extracted and microscopic slides prepared and then identified. In this study five species were identified of which two spices were new and indicated with asterisk as below. A key to all species of the genus Anoplocheylus is also provided. Pseudocheylidae: Anoplocheylus malayeriensis Ueckermann & Khanjani, 2004; Anoplocheylus tauricus Livshitz & Mitrofanov, 1970; Anoplocheylus bonabjadidiensis Navaei- Bonab, 2011; Anoplocheylus sp.1*; Anoplocheylus sp.2*

Keywords:*Acari, Pseudocheylidae, Anoplocheylus, new species, Iran*

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PS1M103

Taxonomy

P1

New detection of entomopathogenic fungi from the Sudan

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Entomopathogenic fungi are important biocontrol agents worldwide. Recent studies in Sudan have revealed numerous promising isolates. Fortuitously, an infected dead beetle showing dense fungal growths was encountered during 2010/11 on Lablab purpureus at El-Gurair, Northern Sudan. It is an unidentified pest of L. purpureus. Therefore, the sample was studied to identify the pathogenic agents. Part of fungal growths was cultured on PDA media and incubated in the dark at 240C. The variable growths obtained were purified, prepared on slides and studied for micro-morphological features. Based on literature, many fungi were identified (viz., Alternaria sp., Penicillium sp., Aspergillus sp., Paecilomyces sp. and Beauveria bassiana), but the latter two were stressed as important bioagents. The first one was characterized by white dense colony, slower growing, no zonation and white lower surface. Under (X100), dense clustered conidiogenous cells, flask shaped, with extended zigzag apexes, besides one celled hyaline globose conidia were seen. Moreover, a bioassay of this fungus against Epilachna elaterii has reflected the characteristic white muscardine sign. Such features suggested the detection of B. bassiana. However, the other fungus showed fast growing flat colony, powdery, initially white then turn brown all-round. Under (X40), well developed conidiophores bearing whorls of divergent branches ending with hyaline phialides were appeared. The conidia were ovoid, one-celled, with long dry chains. These features put this fungus under the genus Peacilomyces. So, the above two fungi were considered as first records in Sudan. They necessitate further studies to be evaluated in formulating biopesticides.

Keywords:*Entomopathogenic fungi, Beauveria bassiana, Paecilomyces sp., Northern Sudan.*

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PS1M104

Taxonomy

P1

The physical therapy taxonomy

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[1]New Era, Nigeria, [2]New Era, Nigeria

Background/Objective: Outcomes research is in need of a classification system of physical therapy (PT) interventions for acute traumatic spinal cord injury (SCI) rehabilitation in the United States. The objective of this study was to describe a taxonomy (system to categorize and classify interventions) to examine the effects of PT interventions on rehabilitation outcomes. Methods: The SCIRehab study uses the rigorous observational practice-based evidence methodology to examine current treatment processes without changing existing practice. PT clinicians and researchers from 6 centers developed a taxonomy to describe details of each PT session. Results: The PT taxonomy consists of 19 treatment activities (eg, bed mobility, transfers, wheelchair mobility, strengthening and stretching exercises) and supplementary information to describe the associated therapeutic interventions. Details that focus on patient assistance needs and family involvement are included as additional descriptors to help to describe and justify PT activity selection. Time spent on each activity is used as the measure of intensity. Conclusion: The detailed PT taxonomy documentation process, which offers efficiency in data collection, is being used for all PT sessions with 1,500 patients with acute traumatic SCI at the 6 participating centers. It might be the first attempt to document the many details of the PT rehabilitation process for patients with SCI in the United States.

Keywords:*Spinal cord injuries, Rehabilitation, physical, Physical therapy, Taxonomy, Practice-based evidence,*

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PS1M105

Taxonomy

P1

An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea

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[1]New Era, Nigeria, [2]New Era, Nigeria

Reference phylogenies are crucial for providing a taxonomic framework for interpretation of marker gene and metagenomic surveys, which continue to reveal novel species at a remarkable rate. Greengenes is a dedicated full-length 16S rRNA gene database that provides users with a curated taxonomy based on de novo tree inference. We developed a 'taxonomy to tree' approach for transferring group names from an existing taxonomy to a tree topology, and used it to apply the Greengenes, National Center for Biotechnology Information (NCBI) and cyanoDB (Cyanobacteria only) taxonomies to a de novo tree comprising 408315 sequences. We also incorporated explicit rank information provided by the NCBI taxonomy to group names (by prefixing rank designations) for better user orientation and classification consistency. The resulting merged taxonomy improved the classification of 75% of the sequences by one or more ranks relative to the original NCBI taxonomy with the most pronounced improvements occurring in under-classified environmental sequences. We also assessed candidate phyla (divisions) currently defined by NCBI and present recommendations for consolidation of 34 redundantly named groups. All intermediate results from the pipeline, which includes tree inference, jackknifing and transfer of a donor taxonomy to a recipient tree (tax2tree) are available for download. The improved Greengenes taxonomy should provide important infrastructure for a wide range of megasequencing projects studying ecosystems on scales ranging from our own bodies (the Human Microbiome Project) to the entire planet (the Earth Microbiome Project). The implementation of the software can be obtained from

Keywords:*evolution, phylogenetics, taxonomy*

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PS1M106

Taxonomy

P1

Faunal notes of family Arctiidae (Lepidoptera, Noctuoidea) from Korea

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The family Arctiidae belong to the superfamily Noctuoidea, with more than 11,155 named species, 750 genera all over the world (Heppner, 2005), small to large size, often quite robust and colorful, it was divided into three subfamilies: Lithosiinae, Arctiinae, and Syntominae (Kitching and Rawlins, 1999; Conner, 2009). The first Korean fauna of Arctiidae was published by Butler (1882), and he reported one species, Stigmatophora micans (Bremer et Gray). Arctiidae was mainly studied by a few foreign entomologists. After 1965, Korean entomologists began to research in this family. The taxonomic history of the Korean Arctiidae has recently revised by Park (2000), and he reported 75 species belonging to 31 genera including North Korea. Many checklists and references have been published about Arctiidae of Korea, we reviewed these references including our collection data. In this study, total of 63 species of 30 genera belonging to 2 subfamilies: Lithosiinae and Arctiinae in South Korea. Among them, one species is newly recorded, and one species has not been identified in genus Eilema from South Korea.

Keywords:*Lepidoptera, Arctiidae, taxonomy, new record, Korea*

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PS1M107

Taxonomy

P1

Biotaxonomy of the family Cicadidae from Vietnam(Hemiptera: Auchenorrhyncha)

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Vietnam is one of the world's hot spots of biodiversity, reflected in its great diversity of landscapes and ecosystems, partly depending on the long and diversely structured coastline and the wide range of latitudes and altitudes of the country. Cicadas are ecologically important because they are the major root-feeding herbivores in tropical forests and also serve as a food source for birds and other insectivorous vertebrates (including humans!). Because of their multi-year life-cycles and dependence on mature, intact forest habitats, they have also proven to be very valuable as indicators of forest habitat integrity. Faunal studies on cicadas in this region would also contribute to our understanding of general mechanisms that have generated high biodiversity in the region. All three subfamilies of Cicadidae, the Cicadinae, Cicadettinae, and Tettigadinae, are now known to be represented in Vietnam. A total of 132 species and 47 genera of Cicadidae from Vietnam were taxonomically revised. There are 16 species, 7 genera, and 4 tribes of Cicadettinae, 115 species, 39 genera, and 7 tribes of Cicadinae, and 1 species of Tettigadinae respectively. The relative percentage of the species richiness in Cicadinae (87.12%), Cicadettinae (12.22%) and Tettigadinae (0.76%) respectively conducted based on the revision work. Presented here, we have identified one genus new to science, which belong to subfamily Cicadinae. Eight species: Platypleura mira Distant, 1904, Oxypleura calypso Kirby, 1888, Gaeana laosensis Distant, 1917, Gaeana vestita Distant, 1905, Haphsa fratercula Distant, 1917, Haphsa opercularis Distant, 1917, Macrosemia divergens (Distant, 1917), Macrosemia assamensis (Distant, 1905) are considered

Keywords:*Cicadas, resources, fauna, distribution, Vietnam*

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PS2M108

Physiology

P2

Different physiological roles of two dopamine receptors in isolated salivary glands of the blacklegged tick, *Ixodes scapularis*.

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Ticks are obligatory ectoparasites of many vertebrate transmitting pathogens causing diseases including the Lyme disease. Tick salivary secretion is crucial not only for successful feedings for injection of bioactive salivary components into the host but also for osmoregulation after the ingestion of large amount of blood. Control of the salivary glands involves dopamine that is the most potent salivation inducer. We previously have identified two dopamine receptors expressed in the salivary glands and named as dopamine receptor (D1) and dopamine-like receptor (D1L) based on their sequence similarities to the vertebrate dopamine receptors. We established heterologous functional assays of each receptor for pharmacological characterizations. We have identified one D1 specific agonist (SKF82958) and two D1L specific antagonists (acepromazine and fluphenazine) that pharmacologically discriminate the two receptors. Isolated tick salivary glands were examined for measuring the secretory activities after pharmacological treatments. Increases in the volume of individual acinus of the salivary glands were measured over time after the treatment. Based on the results from our studies using receptor-specific antagonists and agonists, dopamine triggers two different physiological actions through two respective receptors. We propose that high-sensitive receptor D1 triggers fluid transport in the epithelial cells of the acini and low-sensitive receptor D1L is involved in pumping actions of the acini. Understanding the mechanisms of tick salivary gland control will allow us to develop the novel method for disruption of tick feeding.

Keywords:*salivary gland, salivation, secretion, dopamine receptors, pharmacological characterization*

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PS2M109

Physiology

P2

Cuticular lipids, water loss and desiccation resistance changes in *Z. indianus* and *D. melanogaster*

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We investigated within as well as between population variability in desiccation resistance, cuticular lipid mass per fly and cuticular water loss in nine geographical populations of a tropical drosophilid, Zaprionus indianus. Interestingly, the amount of cuticular lipids and desiccation resistance in this non-melanic species are significantly higher as compared with melanic Drosophila melanogaster. On the basis of isofemale line analysis, within population trait variability in cuticular lipid mass per fly is positively correlated with desiccation resistance and negatively correlated with cuticular water loss but show lack of correlation with body size. We observed geographical variation in the amount of cuticular lipid mass per fly in Zaprionus indianus but no such divergence was found in D. melanogaster. In both the species, geographical variations in desiccation resistance are negatively correlated with cuticular water loss but the underlying mechanisms for changes in cuticular permeability are quite different. Thus, we may suggest that body melanisation and cuticular lipids may represent alternative strategies for coping with dehydration stress in melanic versus non-melanic drosophilids. For both the species, desiccation resistance and cuticular water loss are correlated with regular increase in aridity in the northern subtropical localities as compared with southern peninsular humid tropical localities.

Keywords:*cuticular lipids, cuticular water loss, Zaprionus indianus*

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PS2M110

Physiology

P2

Molecular characterization of the cDNA encoding chitinase and its expression in oriental fruit fly Bactrocera dorsalis

Wen-Jia Yang[1], Kang-Kang Xu[2], Wei Dou[3], Jin-Jun Wang[4]

[1]Southwest University, China, [2]Southwest University, China, [3]Southwest University, China, [4]Southwest University, China

Insect chitinases belong to a large and diverse family of hydrolytic enzymes, which are responsible for chitin degradation in the cuticle and peritrophic matrix (PM) during the insect molting. In this study, we have identified a cDNA putatively encoding a chitinase and named it as BdCht2 (GenBank accession number: JN100105) in the oriental fruit fly Batrocera dorsalis. The BdCht2 gene consists of four exons and three introns and encodes peptide of 483 amino acid residues, which was composed of a singal peptide, a chitin active site, a linker region and a chitin-binding domain. Phylogenetic analysis suggested that BdCht2 is orthologous to the Cht2 proteins identified in other insect species. Real-time quantitative PCR showed that BdCht2 was expressed at all tested developmental stages, and expressed at highest level in the third larva stage. Furthermore, the BdCht2 transcript was predominately found in larva midgut. The results support the hypothesis that BdCht2 is responsible for digesting the chitin associated with the PM in epithelial cells of the midgut. Further studies will be necessary to elucidate the biological functions of BdCht2 gene in the oriental fruit fly. By using a feeding- based RNAi technique, we will detect the BdCht2 transcript level of the larva midgut and chitin content in the PM as compared with the control larvae fed on the diet containing GFP dsRNA. The results about the effect of BdCht2 knock down on midgut chitin metabolism can provide evidence that BdCht2 whether affects the growth and developmental of the larvae B. dorsalis.

Keywords:*Bactrocera dorsalis, Chitinase, Midgut, Real-time quantitative PCR*

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PS2M111

Physiology P2

Molecular mechanisms of acaricides resistance in *Panonychus citri* (Acari: Tetranychidae)

Tian-Bo Ding[1], Kun Zhang[2], Wei Dou[3], Jin-Jun Wang[4]

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The citrus mite, Panonychus citri (McGregor) is the most common and important spider mite pest of citrus orchards in China. Chemical spraying and several biological/ecological traits of P.citri that make it develop resistance rapidly to acaricides. As a result, P. citri become the third species that develop severe resistance from the family Tetranychidae. Our results showed that the significant correlation between GSTs activity and the LC50 of pyridaben existed, suggested that GSTs could be associated with varying susceptibility levels to pyridaben. After selection with abamectin and fenpropathrin in the laboratory, activities of detoxification enzyme, CarE was related to these two acaricides resistance. After induced by four different acaricides (abamectin, azocyclotin, pyridaben and spirodiclofen), the expression levels of two P450 genes, cyp4cf1 and cyp4cl2, both increased. In addition, six GSTs genes and 11 CES genes were cloned and the expression profiles were analyzed. An unprecedented 4 gigabase pairs cDNA library including four developmental stages of P. citri (eggs, larval, nymphal, adult) was constructed by using the cost-effective short read sequencing technology (Illumina). A total of 132 unigenes (composed by 79 of P450s, 24 of GSTs, 13 of CES, 1 of AChE, 1 of VGSC, 10 of nAChR, 3 of GABA receptor and 1 of Cytb) which could be associated with acaricides resistance were identified.

Keywords:*Panonychus citri, resistance mechanism, detoxification enzymes, transcriptome*

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PS2M112

Physiology

P2

A quantitative assay for juvenile hormones and their precursors using fluorescent tags

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The lipophilic character of JH, its low concentration, fragility and the tendency to bind nonspecifically to everything had made difficult its quantification. A sensitive and robust new method was developed to quantify JH and its precursor pools in biological samples. The assay is based on the derivatization of JH with fluorescent tags, with subsequent analysis by reverse phase HPLC and a fluorescent detector (HPLC-FD). Linear responses were obtained over the range of 10-20 to 1000 fmols. Opening of the epoxide ring converts JH III into a diol form that is more hydrophilic and less adsorbed onto surfaces (less "sticky"). We successfully tagged the carboxyl groups of 5 precursors hydroxymethylglutaryl-CoA, mevalonate, mevalonate phosphate, mevalonate diphosphate and farnesoic acid (FA); the thiol groups of acetyl-CoA and acetoacetyl-CoA, the hydroxyl group of farnesol and the aldehyde group of farnesal. All these compounds were detected in mosquito corpora allata-corpora cardiaca extracts (CA-CC). FA and JH III levels were reproducibly quantified from CA-CC, hemolymph and whole body of female adult mosquito (in addition, JH III and JH III bisepoxide were detected in whole body of adult Drosophila melanogaster). The HPLC-FD protocol is simple, fast and relatively inexpensive; when quantifying JH from hemolymph or CA samples, the total time needed to extract the sample, open the ring and derivatize the sesquiterpene was less than 90 min and multiples samples can we processed simultaneously. Quantification from whole body samples is more laborious and takes around 6h.

Keywords:*Juvenile hormone, method, quantification, fluorescence*

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PS2M113

Physiology

P2

Effects of thyroxin and potassium iodid on biological and physiological paramerters of the mulberry silkworm, *Bombyx mori* L (Bombycidae: Lepidoptera)

Gadelhak Gaber Gadelhak[1], Ibrahim A. Rawash[2], Abir A. Gad[3], Mamdouh H. Idress[4]

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Thyroxin and potassium iodid have been used as suplementary nutrients for the mulburry silkworm, Bombyx mori L. Ascending concentrations were applied to mulberry leaves as a source of food at the last larval instar. Treatments with potassium iodide at concentrations ranged from 10 to 75 ?g/ml significantly increased the mean weight of mature larvae, pupae, cocoon shells as well as silk glands. The cocoon shells weight and mean number of deposited eggs/female increased by about 31.7 and 32.8%, respectively, when larvae were fed on mulberry leaves treated with 50 ?g/ml potassium iodide. Results indicated that all tested concentrations of thyroxin (10 to 50 ng/ml) significantly increased the mean weight of mature larvae, pupae and cocoon shells. The use of thyroxin at the concentrations 100 ng/ml increased silk and eggs production by about 33.9 and 23.99%, respectively. The tested compounds had a positive effect on the silkworm CA activity and the total haemolymph proteins.

Keywords:*Bombyx mori, silk production, haemolymph, thyroxin, potassium iodide*

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PS2M114

Physiology

P2

Effects of insulin as a peptide hormone and estradiol as a steroid one on physiological and biological parameters of the silkworm, Bombyx mori L (Bomycidae: Lepidoptera)

Gadelhak Gaber Gadelhak[1], Ibrahim A. Rawash[2], Abir A. Gad[3], Mamdouh H. Idriss[4]

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Effects of Insulin on B. mori larvae was studied on the last larval instar that was injected with doses ,0.01, 0.05, 0.1 and 0.4 IU/larva. Doses increased the mean weights of mature larva, pupa, silk gland, cocoon shell and number of deposited eggs/female. The moderate dose of Insulin, 0.05 IU/larva increased the mean weights of larva, pupa, silk gland, cocoon shell by about 27.3%, 29.7, 23.2 % and 19.08 % respectively. Also, the same dose caused an increase in the number of deposited eggs/female by about 42.6 %. Moreover, the treatment with 0.05 IU/larva caused an increase in the total hemolymph proteins by about 37.5%. The same dose of insulin increased the CA surface area by about 36.7% more than control. The effect of estradiol on the last larval instar of B. mori at concentrations of 50, 100, 250 and 500 ?g/L was studied. The mean weights of mature larva, pupa, silk gland and cocoon shell have been significantly increased. Estradiol at the rate of 100 ?g/L increased the mean weights of larva, pupa, silk gland by about 45.5%, 20.2%, 55.3% and 29.8% respectively. The same concentration caused an increase in the number of deposited eggs per female. It als, increased the total hemolymph proteins. Moreover, the treatment with estradiol caused an increase in the CA surface area by about 43.5% more than control

Keywords:*Bombyx mri, Insulin, Estradiol, silk production*

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PS2M115

Physiology

P2

Are microsatellite mutation rates higher in Orthopterans?

Marie-Pierre Chapuis[1], Christophe Plantamp[2], Rejane Streiff[3], Laurence Blondin[4], Gregory Sword[5]

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Much remains to be learned about the mutational processes governing the evolution of microsatellite repeat regions. Empirical data on microsatellite mutation rates in insect taxa is notably lacking. Here we first analyzed microsatellite within-population diversity compiled from the literature. This survey of 210 insect species from six major orders revealed that within Orthopterans levels of genetic diversity were ~20% higher and microsatellite repeat arrays were longer than in any other group. Because of the mutation dependence on repeat length, this result suggests a higher microsatellite loci mutation rate in the Orthoptera. We then tested empirically this hypothesis in the Desert locust, Schistocerca gregaria, by estimating germline mutation rates at 10 microsatellite loci from pedigree data.

Keywords:*microsatellite, mutation rate, Orthoptera, repeat number*

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PS2M116

Physiology

P2

Phylogeography of the walnut twig beetle, *Pityophthorus juglandis* Blackman, the insect vector of Thousand Cankers Disease, in the United States

Paul Rugman-Jones[1], Richard Stouthamer[2], Andrew D. Graves[3], Steven J. Seybold[4]

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Over the past decade, widespread dieback of walnuts (Juglans spp.) in the western United States, has been attributed to Thousand Cankers Disease (TCD). TCD results from aggressive feeding in the phloem by the walnut twig beetle (WTB), Pityophthorus juglandis, accompanied by inoculation of its feeding galleries with a pathogenic fungus, Geosmithia morbida carried on the surface of the beetle, instigating canker development in the phloem. Symptomatic trees typically die within 3 years. WTB has a long collection history in the southwestern U.S. and northern Mexico (collected in 1896, New Mexico; 1907, Arizona; 1959, California; 1960, Mexico). However, WTB has not historically been considered a major pest of walnut trees and TCD appears to be a new phenomenon. Since the early 1990's, the distribution of WTB has expanded, accompanied by the threat of TCD. WTB is now known from the western U.S. states of California, Oregon, Washington, Nevada, Utah, Arizona, New Mexico, Colorado, and Idaho. More significantly, WTB (and TCD) has now been detected in the eastern U.S. states of Tennessee (2010), Virginia (2011), and Pennsylvania (2011), placing the disease in direct proximity to highly valuable (> $500 billion) native timber stands of eastern black walnut, Juglans nigra. Using mitochondrial DNA we examined variation among over 60 U.S. populations of WTB and found evidence of two clearly divergent lineages. Little geographic overlap of these lineages occurs, but parapatric populations exist in Colorado, New Mexico, and Utah. The specific status of WTB, and potential phylogeographic and evolutionary origins of TCD are discussed.

Keywords:*cryptic species, COI, Juglans spp., fungal pathogen, biological invasion*

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PS2M117

Physiology

P2

Transcriptomic analysis of oocyte resorption after hydric stress in the German cockroach

Maria Dolors Piulachs[1], Anibal de Horna[2], Alba Herraiz[3], Xavier Belles[4]

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Water stress impairs reproduction on insects, causing oocyte resorption in females. The present communication describes the molecular changes underlying oocyte resorption that occur in the German cockroach, Blattella germanica, after water deprivation. We suppressed drinking water to B. germanica females on day three of the adult life, and fluorescence microscopy showed that ovarian resorption started between 1 and 2 days later, as evidenced by a severe disorganization of the cytoskeleton. To identify genes that might be involved in oocyte resorption we carried out RNA ultrasequencing and obtained two respective transcriptomes from ovaries of five-day-old females: one from control specimens and the other from females that were water-deprived two days earlier. The comparative analysis of these two transcriptomes revealed that sequences that are differential expressed, with a p-value of 0.999, are mostly assigned to a Gene Ontology terms related with structural cell components. Transcripts related with processes of proteolysis and engulfment are up-regulated, whereas those involve in cell components organization are down-regulated in the ovaries from water-deprived females, which highlight the state of cellular disintegration involved in resorption. Further steps of this study would be to select the most significant transcripts, to validate the quantitative changes by qRT-PCR, and to carry out RNAi studies to assess their functions.

Keywords:*Blattella germanica, oogenesis, panoistic ovary*

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PS2M118

Physiology

P2

Kruppel homolog 1 and metamorphosis in the German cockroach. Expression, post-transcriptional regulation by microRNAs and biological role

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Endocrinologically speaking, metamorphosis is regulated by ecdysteroids, which induce molts, and juvenile hormone (JH), which repress metamorphic changes. At a molecular level, the action of ecdysteroids has been thoroughly studied in two species, in the highly derived fly Drosophila melanogaster, which is a holometabolous model, and in the less-derived cockroach Blattella germanica, a hemimetabolous species. Conversely, the molecular action of JH is poorly understood, with the bulk of data being available for holometabolous models. Research on the molecular action of JH in hemimetabolous species is therefore needed in order to elucidate the evolution of insect metamorphosis from hemimetaboly to holometaboly. In light of this, we have studied the function of Kruppel homolog 1 (Kr-h1) in B. germanica. Kr-h1 is a Zn finger transcription factor whose function as transductor of the antimetamorphic action of JH has recently been demonstrated in D. melanogaster and in the beetle Tribolium castaneum. The expression studies during the last nymphal instars show that Kr-h1 is suddenly down-regulated 24 h after the molt to the last instar nymph, and anti-miR experiments suggest that miR-2/miR-13 family of microRNAs might be involved in such a regulation. Importantly, RNAi experiments indicate that Kr-h1 has a repressor role in the frame of the JH signalling pathway and in the context of metamorphosis, as occurs in D. melanogaster and T. castaneum, thereby suggesting that this role has been conserved from cockroaches to flies, i.e., across some 350 million years of insect evolution.

Keywords:*Kruppel-h1, juvenile hormone, metamorphosis,*

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PS2M119

Physiology

P2

The microRNA let-7 has a key role in insect vein patterning during wing metamorphosis

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In most insects, the miRNA let-7 clusters with miR-100 and miR-125 in the same primary transcript. The three miRNAs are involved in developmental timing in the nematode Caenorhabditis elegans and in the fly Drosophila melanogaster. In the cockroach Blattella germanica, the expression of these miRNAs peaks in the last instar nymph, before metamorphosis. When let-7 and miR-100 are depleted with specific anti-miRNAs, the resulting adult shows the wings malformed: either reduced in size (anti-miR-100) or with wrong vein patterning (anti-let-7). Depletion of miR-125 induces no apparent effects. In D. melanogaster, many are genes involved in wing maturation, and one of them is the Drosophila serum response factor (DSRF). Towards the end of the last instar larvae, previous to metamorphosis, DSRF expression is down-regulated in the longitudinal veins by Ras-mediated epidermal growth factor (Egfr) signaling. Reduction of DSRF expression seems necessary to reach a right proportion between wing veins and interveins. In B. germanica wing buds, DSRF is down-regulated during the last instar nymph, in parallel to the up-regulation experienced by let-7. We propose that let-7 plays a key role in vein-intervein patterning of B. germanica by targeting DSRF mRNA, thus helping to down-regulate the expression of this gene post-transcriptionally.

Keywords:*microRNAs, let-7, metamorphosis, wing maturation*

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PS2M120

Physiology

P2

Progress of subunit composition studies on nicotinic acetylcholine receptors from *Nilaparvata lugens*, an important rice insect pest

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The subunit composition of insect nicotinic acetylcholine receptors (nAChRs) decided the pharmacology properties of the receptors, such as the effects of point mutations on insecticide resistance. We obtained evidences for the co-assembly of two Nilaparvata lugens nAChR α subunits (Nlα1 andNlα2) with rat β2 subunit into a receptor complex, a functional triplet nAChR (Nlα1/Nlα2/β2), in Xenopus oocytes. We also demonstrated that N. lugens Nlα3 and Nla8 and with rat β2 subunit into another triplet nAChR (Nlα3/Nlα8/β2). Nlα3/Nlα8/β2 was more sensitive to imidacloprid than Nlα1/Nlα2/β2, and the previous identified mutation Y151S in Nlα3 showed more effects on the imidacloprid sensitivity in Nlα3/Nlα8/β2 than Y151S mutation in Nlα1 of Nlα1/Nlα2/β2, which indicated that Y151S mutation in Nlα3 might be more important in contributing to neonicotinoid resistance than the Nla1 Y151S mutation in N. lugens. Previous study revealed that two binding sites for imidacloprid with different affinities in N. lugens. Co-immunoprecipitation studies with native preparations of N.lugens nAChRs demonstrated the co-assembly of three insect subunits (Nlα1, Nlα2 and Nlβ1) into one receptor complex and of Nlα3, Nlα8 and Nlβ1 into another. In native preparations of N. lugens nAChRs, the deletion of [3H]imidacloprid binding by subunit-selective antisera immunoprecipitation indicated that Nlα1, Nlα2 and Nlβ1 (Nlα1/Nlα2/Nlβ1) contributed to a low affinity binding site (Kd=1.5±0.2 nM) for imidacloprid and Nlα3, Nlα8 and Nlβ1 (Nlα3/Nlα8/Nlβ1) contributed to a high affinity binding site (Kd=3.5±0.6 pM).

Keywords:*subunit composition, nicotinic acetylcholine receptors, Nilaparvata lugens*

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PS2M121

Physiology

P2

Molecular characterization and functional study on chitin synthase genes from *Locusta migratoria*

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Current knowledge on chitin synthase (CHS), especially their structures, functions, and regulations in insects is still very limited. We characterized two chitin synthase genes, LmCHS1 and LmCHS2, from Locusta migratoria. LmCHS1 and LmCHS2 were predicted to encode proteins of 1,576 and 1,523 amino acid residues, respectively. Their deduced amino acid sequences show high similarities to other insect chitin synthases. Furthermore, two alternatively spliced transcripts of LmCHS1, LmCHS1A and LmCHS1B, were identified. The two variants differ only in one exon consisting of 177 nucleotides. Analysis of expression levels of these transcripts in different tissues and developmental stages indicated that LmCHS1A predominates in the integument during all developmental stages, whereas LmCHS1B is more abundantly present in the tracheae and LmCHS2 was specifically expressed in midgut and gastric caeca during feeding stages. Functional analysis of the two chitin synthase genes revealed unique and complementary roles. The phenotypes resulted from injection of LmCHS1A dsRNA were similar to those from the injection of LmCHS1 dsRNA, insects can not detach old cuticle and molt to the next stadium, whereas the locusts injected with LmCHS1B dsRNA exhibited crimpled cuticle phenotype. However, LmCHS2-specific RNAi had no effect on morphology of locusts, but the average length of midgut is shorter than those from control insects and the formation of well structured peritrophic matrix was disrupted in HE stained LmCHS2 dsRNA-treatment samples. Our results suggest that both chitin synthase genes are essential for insect growth and development and may represent an attractive target site for controlling locusts.

Keywords:*chitin synthase, expression pattern, RNAi, function, Locusta migratoria*

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PS2M122

Physiology

P2

Identification and functional characterization of sex pheromone receptors in two sibling species, *Helicoverpa armigera* and *Helicoverpa assulta*

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Sex pheromones released by female moths are detected with high specificity and sensitivity in the olfactory sensilla of antennae of conspecific males. The two sibling species, Helicoverpa armigera and Helicoverpa assulta, are important agricultural pests around the world. They share the same two main sex pheromone components, Z11-16: Ald and Z9-16: Ald, but with almost reversed ratio. Here we identify the four sex pheromone receptors from the two Helicoverpa species. Functional analyses of the sex pheromone receptors are conducted using heterologous expression in Xenopus laevis oocytes and two-electrode voltage clamp recordings. Meanwhile, we compare expression levels of these genes between the male and female antennae in the two species.

Keywords:*Helicoverpa, sex pheromone, olfactory receptor, Xenopus, two-electrode voltage clamp recording*

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PS2M123

Physiology

P2

Molecular physiology of epithelial water transport in the lepidopteran hindgut

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Aquaporin (AQP), water channel, facilitates first and foremost the transmembrane transport of water molecules and is now recognized as one of the key players in epithelial and cellular osmoregulaton in insects. In the silkworm, Bombyx mori, the movement of water through the epithelia within the cryptonephric rectal complex is likely facilitated by the two AQPs, AQP-Bom1 and AQP-Bom3. Both are functionally water-specific. Phylogenetically, AQP-Bom1 and AQP-Bom3 belong to the DRIP (Drosophila integral protein) and PRIP (Pyrocoelia rufa integral protein) subfamilies, respectively, of the insect AQP clade. AQP-Bom1 [DRIP] was exclusively expressed at the apical plasma membrane of colonic and rectal epithelial cells, whereas AQP-Bom3 [PRIP] was expressed at the basal plasma membrane of these cells. This polarised localisation of DRIP/PRIP was also observed in the outer cryptonephric Malpighian tubules (outer cMT) and in the six tubules just outside the cryptonephric rectal complex (rectal lead MT). In the rectal complex, water is transported from the rectal lumen to the perinephric space and then deposited into the lumen of the outer cMT; the water then goes through the tubular lumen to exit the complex and is finally transported across the rectal lead MT, which is the very limited region of the water-permeable sites in MT epithelia. Two distinct water-specific AQPs as "DRIP: apical ~ PRIP: basal orientations" confer high-capacity water flow with a transcellular manner and function in retrieving water in the epithelial cells of lepidopteran hindgut. This process circumvents unnecessary water loss and protects caterpillars from accidental starvation.

Keywords:*Aquaporin, cryptonephric complex, DRIP, PRIP, Malpighian tubules, excretion, Bombyx mori*

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PS2M124

Physiology

P2

Structure activity relationship of a novel juvenile hormone, JHSB3

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The juvenile hormone (JH) has been expected to be a prime lead molecule for developing a "third-generation pesticide", because of its significant growth regulation effects in insects. Heteroptera includes many agricultural and medical pests. The development of Heteroptera-specific insecticides poses a pivotal subject in this field. We have reported the structure determination of a novel juvenile hormone, JHSB3, isolated from a stink bug, Plautia stali (Kotaki, et al., Org. Lett. 2009, Kotaki, et al, Psyche, 2012, review). In this paper, we would like to describe the structure activity relationship of JHSB3 toward development of Heteroptera-specific insecticides. JHSB3 [methyl (2R,3S,10R)-2,3;10,11-bisepoxyfarnesoate] bears two epoxides, and olefin and methyl ester moieties, on the sesquiterpene skeleton. The JH analogs in which the functional groups of JHSB3 were removed or substituted with other functional groups have been prepared. These analogs were subjected to juvenilizing activity tests using P. stali. The results indicated that the co-existence of the ester carbonyl group and two epoxides was proved to be crucial for the potent juvenilizing activity. Among the tested compounds, we found highly potent analogs in which the olefinic double bond of JHSB3 was saturated. The methoxy analogs in which the 10,11-epoxide moiety was substituted with a methoxy group exerted a moderate juvenilizing activity (Kaihara, , et al., Tetrahedron, 2012).

Keywords:*Juvenile hormone, JHSB3, heteroptera*

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PS2M125

Physiology

P2

Analysis of expression pattern of *Bombyx mori* gustatory receptors in the tissues and single cells of the taste organs.

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Insects distinguish host plants and others making full use of gustatory sense. Therefore, understanding gustatory system of insects is expected to reveal selection mechanism of host, leading to contribution to insect pest control. Taste substances are recognized by insects using gustatory neurons that express 7-transmembrane receptors assigned to insect gustatory receptor (Gr) family. Many groups have been trying to identify ligands of the Grs using heterologous expression system, but few good results were obtained. One possible reason is that Grs do not function as monomeric receptors, but heterogeneous multimers in combination with other members of the Gr family. Therefore, to identify the ligands of the Grs and to understand gustatory system of insects, it is very important to reveal expression pattern of Grs in each gustatory neurons. We tried to investigate expression pattern of Grs in various tissues and single cells of Bombyx mori larvae which eats selectively mulberry leaves. First, RT-PCR analysis of BmGr expression in antennae, labrum, maxillary lob, maxillary palpus and midgut from larvae was performed. Next, expression pattern of Grs in single gustatory neurons innervating sensilla of the gustatory organs were attempted to investigate by RT-PCR in combination with Laser capture microdissection (LCM). Frozen section of the gustatory organs of B. mori which was previously injected with methylene bule were prepared and stained cells were isolated from the section by LCM. Subsequently, BmGrs being present in the collected cells were examined by RT-PCR. In this conference, we will present the results of these experiments.

Keywords:*Gustatory receptor, Bombyx mori*

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PS2M126

Physiology P2

The role of the TOR pathway in the regulation of juvenile hormone synthesis in *Aedes aegypti*.

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Juvenile hormone III (JH III) plays a central role in regulating ovarian development in female Aedes aegypti mosquitoes. The activation of JH synthesis and the previtellogenic development of the ovaries are stimulated only when nutrients are adequate. The protein kinase TOR (Target of Rapamycin) is the central node of a highly conserved signaling network that regulates cell growth in response to nutrients, hormones and stress. We are using several experimental approaches to test the role of the TOR signaling pathway on the transduction of nutritional signals in the mosquito CA. 1) To study the effect of rapamycin (an inhibitor of TOR) and nutrients on phosphorylation of two major downstream targets of TOR: p70S6 kinase and 4E-binding protein in the mosquito CA 2) To evaluate the effect of rapamycin on the levels of JH synthesis and its precursors using high performance liquid chromatography and fluorescent detection (HPLC-FD), 3) To evaluate the effect of RNAi depletion of specific components of the TOR pathway on JH synthesis, and 4) To evaluate the effect of manipulating the TOR signaling on the mRNA levels of the JH biosynthetic enzymes. We aim to demonstrate that the TOR signaling pathway is involved in the nutritional regulation of CA activity in mosquitoes, and reveal how nutritional signal pathways crosstalk to other regulators (such as allatoregulatory peptides) to modulate JH synthesis.

Keywords:*Mosquito, JH, TOR, Corpora allata, Aedes, Nutrition*

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PS2M127

Physiology

P2

The photoresponse of the brown-winged green stinkbug, *Plautia stali*: The daily activity rhythm and phototaxis.

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The brown-winged green stinkbug, Plautia stali, is an important pest of fruit trees in East Asia, including Japan. However, little is known of its daily activity rhythm and phototactic behavior. This study investigated the daily distribution of locomotor activity in its natural environment and the wavelength dependence of its phototactic behavior at night. Insects were collected in an orchard in Tsukuba and progeny until the fifth generation were used for the experiments. The adults were reared individually in plastic dishes and the locomotor activity was recorded continuously for 1 week in a cypress grove. Under natural light, P. stali had morning and evening activity peaks. The greatest activity was in the 3~4 hours after sunset (dusk to midnight). We also performed a trajectory analysis of the phototactic behavior (attraction to a monochromatic light-emitting diode (LED)) in a wide open-field arena. Phototactic behavior was evoked toward dim light (1×10^15 photons/m2/s) and the animals were attracted to a wide range of wavelengths (from ultraviolet (UV) to red). The most attractive wavelengths were 365 nm (UV) and 525 nm (green). We discuss the adaptive importance of the photoresponse behavior of P. stali.

Keywords:*Circadian rhythm, Phototaxis*

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PS2M128

Physiology

P2

Mode of binding of juvenile hormone binding protein with ligands

Takahiro Shiotsuki[1], Rintaro Suzuki[2], Zui Fujimoto[3], Wataru Tsuchiya[4], Mitsuru Momma[5], Akira Tase[6], Mitsuhiro Miyazawa[7], Toshimasa Yamazaki[8]

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Juvenile hormones (JHs) are involved in many aspects of physiology in development and reproduction. The regulation of JH titer is crucial to normal development in insects. JH titer is mainly regulated by the control of its synthesis and degradation. Also, hemolymph JH binding protein (JHBP) is known for its importance to transfer JH to peripheral tissues in Lepidoptera. The structural mechanism of JH delivery by JHBP was analyzed based upon the crystal and solution structures of apo- and JH-bound JHBP of silkworm. JHBP has two hydrophobic pockets at both ends of the molecular structure, and only one of those is found for JH-binding. JH-binding to the gate-open form results in the fully closed JHBP-JH complex structure where JH is buried completely inside the protein. At the binding site, JH is stabilized by several hydrogen bonds to set an L-shaped form surrounded with several aromatic amino acid residues. JHBP in complex with JH opens the gate helix to release the bound hormone likely by sensing reduction of the dielectric constant at the membrane surface of the target organs. The results obtained reveal the structural mechanism for JH delivery by JHBP in hemolymph, the first essential process of the JH signal transfer.

Keywords:*juvenile hormone, binding protein, structural analysis*

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PS2M129

Physiology P2

Embryonic development of a diving beetle, *Hydaticus pacificus* (Coleoptera, Dytiscidae)

Kazuhiro Niikura[1], Yukimasa Kobayashi[2], Kei Hirasawa[3], Toshio Inoda[4]

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The successive changes of developing embryos of Hydaticus pacificus were described from observations based on light and electron microscopy. The eggs of this species were deposited on water grasses by breeding females in a laboratory. The egg period was about 50 hours under the condition of 28 degree centigrade. The newly laid egg is nearly cylindrical, and is about 2.20 mm long and 0.68 mm wide. As development proceeds, the egg becomes 2.70 mm long and 0.85 mm wide in maximum. The chorion is smooth and thin, and almost transparent throughout the whole egg surface. The early germ band, or embryo, is long, occupying about four fifths of the egg length, and is formed on the ventral surface of the egg. The embryo then elongates and widens, retaining this superficial position throughout development. The openings of the tracheal invaginations, or spiracles, appear in the body wall of the mesothoracic, metathoracic, and the first eight abdominal segments. The spiracles except for those of the eighth abdominal segment, however, close and disappear at late embryonic stages, and the spiracles in the eighth segment remain as those of the newly hatched larva. At the abdominal end the ninth and 10th abdominal segments fuse, and thus the abdominal region consists of nine segments, although the ninth or last segment is highly reduced. Urogomphi appear as a pair of slender projections at the abdominal end. Pleuropodia are formed in the first abdominal segment, but no appendage-like projections appear in other abdominal segments.

Keywords:*Hydaticus pacificus, embryo, egg, pleuropodium, urogomphus*

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PS2M130

Physiology

P2

Accumulation of antidiabetic iminosugars in silkworms reared on mulberry leaves

Masatoshi Hori[1], Shigeto Komatsubara[2], Shinji Onose[3], Oki Higuchi[4], Teruo Miyazawa[5], Kiyotaka Nakagawa[6]

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1-Deoxynojirimycin (DNJ), a constituent of mulberry, is a potent α-glucosidase inhibitor expected to be useful in the diet of diabetic patients. In this study, we compared the DNJ concentrations in 5 silkworm tissues, hemolymph, skin, gut, silk gland, and gut content, and investigated concentration changes during the growth of silkworms. Concurrently, we also investigated 2 other iminosugars, 2-O-α-D-galactopyranosyl-DNJ (GAL-DNJ) and fagomine. These iminosugars possess some α-glucosidase inhibitory activity or have other unique effects. The highest concentrations of DNJ and fagomine were obtained from 2-day-old third-instar larvae, and the concentrations decreased as development progressed. GAL-DNJ concentrations were very low in all developmental stages. Among the silkworm tissues, hemolymph contained the highest concentrations of DNJ and fagomine. The DNJ concentrations in hemolymph were extremely high, reaching approximately 10-fold higher than that of the entire silkworm body. The GAL-DNJ concentration in the gut content was the highest among the silkworm tissues, but the concentration was lower than that of mulberry leaves. These results suggest that when silkworms receive iminosugars from mulberry leaves, DNJ and fagomine are absorbed in intact forms into the hemolymph. However, GAL-DNJ is absorbed in a degalactosyl form into the hemolymph. Furthermore, we analyzed the iminosugar concentrations in the larvae of 2 other mulberry-feeding moths, Hyphantria cunea and Thanatarctia imparilis. The concentrations of these 3 iminosugars in both moth larvae were much lower than those in mulberry leaves. It was believed that these larvae immediately excreted these iminosugars without accumulating them. The accumulation of iminosugars may be specific to silkworms.

Keywords:*silkworm, mulberry, 1-deoxynojirimycin, iminosugar, fagomine, 2-O-α-D-galactopyranosyl-DNJ, diabetes, antidiabetic*

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PS2M131

Physiology

P2

Digestive environment in the midgut of the xylophagous higher termite, *Nasutitermes takasagoensis*

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The xylophagous higher termite Nasutitermes takasagoensis is though to digest cellulose primarily in the midgut. To further explore the detailed cellulolytic system in the midgut of N. takasagoensis, we performed immunohistochemistry and digital light microscopy to determine distributions of cellulolytic enzymes in the midgut as well as the luminal volume to estimate the total cellulolytic activity in the midgut. Although cellulolytic enzymes were uniformly produced in the midgut epithelium, the concentration of endo-β-1,4-glucanase activity and luminal volume in the midgut were comparable to those of the wood-feeding lower termite Coptotermes formosanus, which digests cellulose with the aid of hindgut protists. However, the size of ingested wood particles in N. takasagoensis was considerably larger than that in C. formosanus. Nevertheless, it is possible that the cellulolytic system in the midgut of N. takasagoensis hydrolyzes highly crystalline cellulose to a certain extent. To understand a variety of molecules involved in the digestive system in the midgut, we performed a trascriptome analysis with a pyrosequencer, 454 GS junior using mRNA extracted from the midgut. So far, no mRNAs encoding lignocellulolytic enzymes other than β-glucosidases belonging to the glycoside hydrolase family 1 (GH1) and endo-β-1,4-glucanases affiliated with GH9 have been identified from the midgut transcriptome. On the other hand, in addition to cellulases, the midgut abundantly contains mRNAs that encode chitinases and proteinases, which might play roles in degradation of fungal commuity on the surface of ingested wood particles and/or of termite bodies ingested through cannibalism.

Keywords:*cellulase, digestion, transcriptome*

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PS2M132

Physiology

P2

Substrate vibrations mediate avoidance behavior via the femoral chordotonal organ in a cerambycid beetle, *Monochamus alternatus*

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Most insects can detect substrate vibrations in order to recognize predators and conspecifics. However, little is known about how coleopteran insects respond to vibrations. We investigated vibrational responses and associated sense organs in the Japanese pine sawyer beetle, Monochamus alternatus (Coleoptera: Cerambycidae). This beetle showed behavioral responses, such as startle responses and stridulation from a stationary position, to vibrations with low frequencies below 1 kHz. We identified for the first time the femoral chordotonal organ (FCO) of a coleopteran species. The main body of the FCO contained multiple sensory neurons and was distally connected to the proximal tibia via an apodeme. We subsequently showed that beetles with ablated FCOs did not freeze in response to low-frequency vibrations during walking whereas intact beetles froze. These results indicated that the FCO is responsible for detecting low-frequency vibrations and mediating the freezing behavior. We discuss the behavioral significances of vibrational responses in M. alternatus with respect to predator avoidance and conspecific recognition.

Keywords:*behavior, vibration, sense organ, predator*

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PS2M133

Physiology

P2

Developmental expression profiles of sex-related genes and RNA interference of transformer in *Bactrocera dorsalis*

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Bactrocera dorsalis (Hendel) is an important insect pest that needs to be effectively controlled in Taiwan. To understand more about its sex-specific regulation, we analyzed transformer (tra), transformer-2 (tra-2), doublesex (dsx), and yolk protein gene (yp1) in this oriental fruit fly using RT-PCR. Expression patterns of these genes fluctuated during different developmental stages. High levels of Bdtraf were present in both unfertilized and fertilized eggs. In both larval and pupal stages, the expression of these sex-related genes was relatively low in comparison with that in adult stage. After eclosion, the expression of Bdtraf, Bddsxf and Bdyp1 in female and the expression of Bddsxm in male increased gradually. These phenomena imply that these gene products may have critical physiological functions in sex differentiation pathways. For Bdtra functional assay using RNA interference (RNAi), double-strand RNA covering exon 2 to exon 4 was prepared and injected into 68 early pupae. Among the 19 females that emerged, seven showed deformed ovipositors without egg laying, yet normal ovaries were found upon dissection. All RNAi treated males that emerged had normal external genital organs and spermaries. These observations support that sex determination occurs in early developmental stage. Repressing Bdtra at pupal stage would subsequently produce similar effect on the downstream Bddsx, leading to morphological changes in females.

Keywords:*Bactrocera dorsalis, transformer (tra), transformer-2 (tra-2), doublesex (dsx), yolk protein-1 (yp1)*

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PS2M134

Physiology

P2

Cannibalism modifies metabolic fingerprint and growth rate in *Helicoverpa armigera*

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Cannibalism is known in many insect species, yet its impact on insect metabolism has not been investigated in detail. This study assessed the effects of cannibalism on the metabolism of 4th-instar larvae of Helicoverpa armigera. Two groups of larvae were analyzed: one group fed with artificial plant diet, the other group fed with 4th-instar larvae of H. armigera. Water-soluble organic compounds present in each group were analyzed using one- and two-dimensional nuclear magnetic resonance (NMR). Multi-element analyses of each group were carried out by inductively coupled plasma/optical emission spectrometry. Chemical fingerprints analyzed using principal component analysis (PCA) were statistically different between cannibals and herbivores. In the case of NMR data, a list of compounds potentially present in each sample was produced by matching signals with a database of authentic compounds. Cannibalism reduced larval size and PCA showed that some NMR signals contributed more than others to the total variance of the chemical fingerprints. To identify essential nutrients for the development of larval stages of H. armigera, future experiments are proposed to evaluate if deprivation of these compounds can explain the small size of cannibals.

Keywords:*Cannibalism, Helicoverpa armigera, ICP, OES, insect metabolomics, NMR.*

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PS2M135

Physiology

P2

Sex pheromone desaturase functioning in a primitive *Ostrinia* moth is cryptically conserved in congener's genomes

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(E)-11- and (Z)-11-tetradecenyl acetate are the most common female sex pheromone components in Ostrinia moths. The Δ11-desaturase expressed in the pheromone gland (PG) of female moths is a key enzyme that introduces a double bond into pheromone molecules. A single Δ11-desaturase of Ostrinia nubilalis, Onub Z/ E11, has been shown to produce an ~7:3 mixture of (E)-11- and (Z)-11-tetradecenoate from the substrate tetradecanoate. In contrast, the sex pheromone of Ostrinia latipennis, a primitive species of Ostrinia, is (E)-11-tetradecenol. This pheromone is unique in that it is not acetylated, and includes no Z isomer. In the present study, through the cloning and functional analysis of a PG-specific Δ11-desaturase in O. latipennis, we showed that the absence of the Z isomer in the pheromone is attributable to the strict product specificity of the Δ11-desaturase in this species, LATPG1. Phylogenetic analysis revealed that LATPG1 was not closely related to Onub Z/ E11. Rather, it was closely related to retroposon-linked cryptic Δ11-desaturases (ezi-Δ11) found in the genomes of O. nubilalis and Ostrinia furnacalis. Taken together, the results showed that an unusual Δ11-desaturase is functionally expressed in O. latipennis, although the genes encoding this enzyme appear to be cryptic in congeners.

Keywords:*Sex pheromone biosynthesis, Δ11-desaturase, retroposon, speciation*

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Physiology

P2

cDNA cloning and mRNA expression of heat shock proteins in *Oxya chinensis*

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Heat shock proteins (Hsps) are a class of quick response proteins when insects are exposed to certain environmental stress. As a molecular marker in ecological toxicology, Hsps is more sensitive than the traditional biological indicators. The rice grasshopper, O.chinensis (Orthoptera: Acridoidea) is one of the important agricultural pests of rice. In this study, four full-length cDNA sequences of hsps genes were acquired from O.chinensis. They were glucose-regulated protein (OcGrp78), heat shock protein 70 (Ochsp70), heat shock protein 90 (Ochsp90), and heat shock protein 40 (Ochsp40), respectively. The mRNA expression in different tissues and developmental stages of rice grasshopper were determined under normal growth conditions in O.chinensis. The results showed that these four hsps appeared differential expression patterns in tissue distribution and during developmental stages. In addition, the expression profiles of these four hsps genes were investigated when insects were treated with Cd. The results suggested that Cd acute injection could induce the expression of OcGrp78, Ochsp70, Ochsp90, and Ochsp40 in O.chinensis, Ochsp70 was the most sensitive response gene when insects exposed to Cd acute stress. The results of chronic Cd exposure resulted in up-regulation of OcGrp78, Ochsp90, and Ochsp40 and down-regulation of Ochsp70. Our results inferred that OcGrp78, Ochsp70, Ochsp90 and Ochsp40 could be used as molecular markers of acute pollution of Cd, whether these hsps genes can be used as molecular markers of chronic Cd pollution remains to be elucidated.

Keywords:*Oxya chinensis, Hsps genes, Cadmium, biomarker*

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Physiology

P2

Transcriptomic analysis of juvenile hormone biosynthetic organ in *Bombyx mori*

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Juvenile hormone (JH) is a lipophilic hormone involved in various aspects such as development, reproduction, and polymorphism. JH is produced from Acetyl-CoA and Propyonyl-CoA in corpora allata (CA) in larvae of silkworm, Bombyx mori. In the JH-biosynthetic pathway, 13 steps of biochemical reaction have been predicted. Ten genes involved in the pathway have been already identified, but three are still uncertain. In order to identify these three genes we performed transcriptomic analysis for corpora cardiaca (CC)-CA complexes using DNA microarray, and compared the results with those of other tissues. In order to cover the transcriptome of CA, we first constructed and sequenced cDNA libraries of CC-CA complex, and clustered the obtained ESTs to establish representative sequences. Using EST clusters as well as predicted genes from B. mori genome, we designed a custom-made 44k DNA microarray for B. mori. The total RNA isolated from CC-CA of day-2 fourth instar larvae, which produce JH actively, and other tissues were analyzed with the microarray. Known JH-biosynthetic genes showed consistent expression with the previous reports, showing the reliability of this study. We found many genes expressed selectively and abundantly in CC-CA complexes. These genes are possibly involved in JH biosynthesis or other CC/CA functions; especially genes encoding for catalytic activities are expected to complete the JH-biosynthetic pathway.

Keywords:*juvenile hormone, JH biosynthesis, DNA microarray, Bombyx mori*

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Physiology

P2

cDNA cloning, thermal regulation and developmental expression of five heat shock protein genes in the western flower thrips, *Frankliniella occidentalis*

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The western flower thrips, Frankliniella occidentalis is a highly invasive pest that is able to exploit many crops across a wide range of environmental conditions. Five full-length cDNAs of heat shock protein (HSP) genes (fo-hsp90, fo-hsp70, fo-hsp60, fo-hsp40 and fo-hsp28.9) were cloned from F. occidentalis and their expression was investigated in relation to cold shock, heat shock, and development using real-time quantitative PCR. All five genes showed high similarity to their counterparts in other species, indicating such genes are highly conserved. The expression of fo-hsps under thermal stress suggests that fo-hsp90, fo-hsp70, and fo-hsp28.9 are both cold- and heat-inducible, fo-hsp40 is only heat-inducible, and fo-hsp60 is thermal-uninducible. For each of the thermal-inducible fo-hsps, the maximal level of induction by heat stress was much higher than that by cold stress. In terms of expression in relation to development, the mRNA level of both fo-hsp90 and fo-hsp60 peaked at stages Larva 1 and Larva 3, yet decreased at stage Larva 2. The moderately high level of fo-hsp40 at stage Larva 1 decreased at stage Larva 2 but then peaked at stage Larva 3. Both fo-hsp70 and hsp28.9 maintained similarly low levels at both stages Larva 1 and Larva 2, and then peaked at Larva 3. However, mRNA level of all five fo-hsps decreased with development of propupae, pupae and adult. These results indicated that, in addition to thermal responses, all the five fo-hsps are involved in development.

Keywords:*Frankliniella occidentalis, heat shock protein genes, expression profile, thermal stress, developmen*

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Physiology

P2

Functional genes for juvenile hormone degradation in insects

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Juvenile hormones (JHs) play important roles in the regulation of a number of physiological processes. JH titer is regulated by the control of its synthesis and degradation. Mainly two groups of enzymes are known to degrade JHs: JH-specific epoxide hydrolase (JHEH) and JH-specific esterase (JHE). To date, functional analysis of jheh- and jhe-related genes is yet carried out. In this study we attempted to evaluate the related genes of jhehs and jhes in two model insects, silkworm Bombyx mori and red flour beetle Tribolium castaneum. The search of the genomic sequence revealed that both species have five or six jheh-related genes. We found that multiple jheh-related gene products in both species could degrade JH efficiently, suggesting that one insect species have multiple JHEHs. Since each jheh-related gene was revealed to be expressed in tissue-specific manner, it is supposed that JHEHs regulate JH titer separately in each tissue. On the other hand, a large number of carboxylesterase genes were identified in the genome of both species: seventy-three for B. mori and forty-eight for T. castaneum. We investigated the enzymatic activity of seven JHE-related proteins in B. mori. However, no protein could degrade JH efficiently besides authentic JHE, suggesting that B. mori has only one JHE. Taking into consideration the previous study that JHEs hydrolyze JH in a reversible reaction whereas JHEHs convert JH to an irreversibly hydrolyzed product, our results indicate that the regulation of JH titer by JHEH and JHE is performed in a distinct manner in the organism.

Keywords:*juvenile hormone epoxide hydrolase, juvenile hormone esterase, genomic analysis, Bombyx mori, Tribolium castaneum*

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Physiology

P2

A new coelomic gregarine parasite from the ghost moth, *Thitarodes pui* (Lepidoptera: Hepialidae)

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Gregarines, a variety of apicomplexan parasites, inhabit the intestine, coelom or reproductive vesicle of invertebrates including insects. Recently, we discovered a coelomic gregarine parasite from Thitarodes pui distributed at 4,156~4,600m in the Tibet Plateau. The ghost moth is known as a host species of entomopathogenic fungus Ophiocordyceps sinensis with medical and economical importance. Visible gregarine gametocysts were found in the coelom of T. pui adult hosts, containing tens of thousands of biconical oocysts. In this work, lots of trophozoites were found in the intestinal lumen of T.pui larvae fed with 10^3 oocysts/larva. Young trophozoites were characterized by a protomerite-deutomerite septum and a flowerlike epimerite. The septum was lost in mature trophozoites (obovoid, 40~100 μm). Morphological characters of oocysts and trophozoites suggest that the gregarine species is closely related to the genus Paraschneideria, which is consistent with previous phylogenetic analysis inferred from SSU rDNA sequences.

Keywords:*coelomic gregarine, Thitarodes pui, parasite, Paraschneideria*

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Physiology

P2

Effects of temperature stress on tropical insects: A case study with the brown planthopper *Nilaparvata lugens* (Stal)

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Nilaparvata lugens (Stal) is the most serious rice pest and widely distributed across Asia. Experiments were carried out to determine the effects of heat stress on nymphs and adults of N. lugens by measuring critical thermal maximum (CTmax), heat coma temperature (HCT) and upper lethal temperature (ULT50). Further experiments investigated the ability of the insect to acclimate at low (15°C) and high temperatures (30oC) compared with the standard rearing regime (23°) by determining critical thermal minimum (CTmin), CTmax, chill coma temperature (CCT), HCT, lower lethal temperature (LLT50) and ULT50. Nilaparvata lugens also showed changes in their thermal tolerance after acclimation. When the rearing temperature was decreased from 23 to 15oC the LLT50 decreased from 2.3±0.4 to 0.5 ±0.4°C in nymphs and from 0.9±0.5 to -2.7±0.4°C for adults. The CTmin was similarly decreased from 15.3±0.3 to 12.5±0.3, 13.1±0.4 to 8.1±0.2 and 12.9±0.4 to 8.8±0.2°C; and the CCT from 6.7±0.2 to 6.2±0.2, 2.1±0.2 to -1.2±0.3 and 2.6±0.2 to -0.3±0.2°C for nymphs and adult females and males. With high temperature acclimation at 30°C the ULT50 increased from 41.8±0.1 to 42.9±0.1 and 42.5±0.1 to 43.6±0.1°C for nymphs and adults, the CTmax from 34.9±1.3 to 37.2±0.1, 37.0±1.0 to 37.3±0.1 and 37.4±0.7 to 37.7±0.1 and the HCT from 37.7±1.3 to 41.0±0.3, 43.4±1.7 to 45.0±0.1 and 42.0±1.8 to 43.2±0.5°C for nymphs and adult females and males. The results indicate that nymphs of N. lugens are currently living at temperatures close to their upper thermal limits, and that acclimation ability is greater at low than at high temperature.

Keywords:*Nilaparvata lugens (Stal), temperature stress, acclimation*

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Physiology P2

The boule gene is required for spermatogenesis in the haploid male of the sawfly, *Athalia rosae*

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Most hymenopterans have haplo-diploid sex determination system in which female is diploid and male is haploid. Diploid females produce haploid eggs with reduction of genome sets, whereas haploid males produce haploid sperm without reduction of genome set. Therefore, maturation divisions similar to mitosis, not meiosis occurs in the spermatogenesis. The boule (bol) gene, a member of the DAZ family gene plays an important role in meiosis in a spermatogenesis-specific manner by regulating translation of the twine (twe: cdc25 homologue gene) mRNA in Drosophila melanogaster (Maines and Wasserman, 1999). Interestingly, the bol homologous gene is found in the genomes of hymenopterans such as Apis melifera and Nasonia vitripennis, though expression and function of this gene is unknown in this group (Schurko et al., 2010). In this study, we examined whether the bol gene is actually expressed and functions during spermatogenesis in haplo-diploid insects using the sawfly, Athalia rosae. First, we cloned and identified two alternatively spliced cDNAs of the bol homologues (Arbol-1, 2), and one cDNA of the cdc25 homologue (Arcdc25). It was revealed by RT-PCR that the Arbol-1 was expressed exclusively in the testis during the maturation division, and Arbol-2 was expressed not only in the testis, but also ovary and somatic tissues. Larval RNAi of Arbol and Arcdc25 resulted in the similar phenotypes: abnormal spermatogenesis and lack of mature sperm. Therefore, our results indicate that the Arbol gene is essential for the spermatogenesis of haploid males in which reductional divisions do not take place.

Keywords:*developmental biology, haplo-diploidy, maturation division, reproduction, spermatogenesis*

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Physiology P2

Production of human beta defensin (DEFB) -1, 2, 3 and LL37 using baculovirus expression system.

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There are many pathogenic microorganisms on human skin, but natural immunity prevents them from invading into the body. Beta-defensin (DEFB) and LL37, antimicrobial peptides, play a role in innate immunity on human epidermis and in maintaining our health. For example, immunosuppressant used for transplant surgery often causes skin disease by decreasing innate immunity. This suppression is a matter of grave concern in medical practice, and it is urgently necessary to develop a technique for preventing skin disease caused by these drugs. DEFB and LL37 are suggested to be the candidate molecules for this purpose. However, it is difficult to express DEFB and LL37 by bacterial protein expression system because they have antimicrobial activity. On the other hand, the antimicrobial activity may not influence protein expression in silkworm-baculovirus expression system. In this study, we constructed 4 distinct expression vectors of antimicrobial peptides, DEFB1, 2, 3 and LL37, respectively and expressed them by silkworm-baculovirus expression system. The recombinant proteins were purified by Ni-affinity chromatography to purify recombinant proteins. Purified recombinant DEFB2 had antimicrobial activity against E. coli and P. aeruginosa. Then their expression levels, yields and activities are compared.

Keywords:*Human beta defensin, LL37, baculovirus expression system, silkworm*

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Physiology P2

Genome-wide analysis for chemoreceptor genes in an insect-phoretic nematode, *Bursaphelenchus xylophilus*

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Chemoreception governs essential aspects of the lives of many animals, including the search for mates and hosts. Animal genomes contain families of genes involved in chemoreception, i.e., olfaction and gustation. The pine wood nematode Bursaphelenchus xylophilus is an endoparasite that causes severe damage to pine forests. The nematode migrates via Monochamus cerambycid beetles. Recently, a high-quality draft genome sequence of the nematode was reported (Kikuchi et al. 2011, PLoS Pathogens). Based on sequence similarity, we investigated B. xylophilus chemoreception genes. Three classes of potential chemoreceptors -gustatory receptors, serpentine receptors, and others- were characterized in B. xylophilus by comparison to chemoreceptors in Caenorhabditis nematodes and insects. The serpentine receptors of B. xylophilus exhibited extensive divergence and duplication. The gustatory receptors represent an unusual chemosensory gene family that share a common origin with insect gustatory receptors; the family has remained small in nematodes but has expanded substantially in insects.

Keywords:*gustatory receptors, serpentine receptors, chemoreception, Monochamus cerambycid beetles*

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Physiology P2

Possible roles of cuticular lipids in mating behavior of *Papilio polytes* (Lepidoptera: Papilionidae)

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Many species of adult butterflies use wing pattern and coloration as the primary cues for mating (sexual recognition and interspecific discrimination), while particular species utilize also male-specific scents. Moreover, they may depend on other sensory cues when inhabiting together with other species possessing similar appearance and odor. Papilio polytes (Papilionidae) inhabits sympatrically with several species of swallowtail butterflies with similar coloration. Before copulation, the male adults were necessarily found to palpate the body and/or wing of mating partners using forelegs. This behavior may be to scrutinize cuticular substances of the partners. The cuticular lipid profiles of 3-d-old adults were compared among P. polytes and its three related species P. protenor, P. macilentus, and P. helenus. GC-MS analyses revealed that the four species had different lipid compositions, in which linear hydrocarbons with 23?31 carbon atoms and hexadecanoic acid were identified as the common major components. P. polytes and P. protenor were sexually dimorphic in lipid composition; however other two species were sexually monomorphic in it. These results suggest that their cuticular lipids serve as chemical signals to express species and sex of swallowtail adults. P. polytes males have two types of sensilla trichodea (LST and MST) on the foreleg tarsi. The presence of a terminal pore in LST and MST indicates these potential functions as gustatory sensilla. Electrophysiological recording clarified that aqueous solutions of cuticular lipids elicited clear responses from half of LST, suggesting that the males could perceive the cuticular lipids of mating partners during palpation.

Keywords:*Adult butterfly, Cuticular lipid, Mating cue, Chemical composition, Gustatory sense*

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Physiology

P2

Effects of eye-removal on the photoperiodic response of the two-spotted spider mite, *Tetranychus urticae*

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The photoperiodic regulation of seasonal events, such as induction or termination of diapause, is important in life cycles of mites. To receive photoperiodic information from the environment, mites must be equipped with photoreceptors. These can be either retinal receptors in the visual system, or nonvisual extraretinal photoreceptors. The predacious mite Amblyseius potentillae, in which ocelli are absent, shows a clear photoperiodic response, clearly indicating involvement of extraretinal photoreceptors in photoperiodism in the mite. In mites equipped with ocelli, however, involvement of retinal photoreceptors in photoperiodism has not been investigated. The two-spotted spider mite Tetranychus urticae possesses two pairs of ocelli. Adult females of this species terminate diapause in response to long days. To see whether the ocellus eyes function as photoperiodic photoreceptors in T. urticae, we removed the eyes with a laser ablation system. When intact mites in diapause were kept under long-day conditions after exposing to a low temperature they terminated diapause, whereas they remained in diapause when they were kept under short-day conditions after low temperature exposure. When both anterior and posterior eyes were bilaterally removed, mites did not terminate diapause even when they were kept under long-day conditions. In contrast, mites showed a clear photoperiodic response in diapause termination, when only anterior eyes on both sides were removed. These results indicate that both anterior and posterior eyes or only posterior eyes function as photoreceptors in photoperiodic termination of diapause in T. urticae.

Keywords:*photoperiodism, photoreceptor, diapause, seasonal adaptation, mite*

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Physiology

P2

The occurrence of cell death mechanisms during follicular atresia induced by nutritional deprivation in *Dipetalogaster maxima* (Hemiptera: Reduviidae), a vector of Chagas' disease.

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In insects, unfavorable physiological or nutritional conditions promote changes in the ovaries, which, in turn, elicit the atresia of some terminal follicles and oocyte resorption. Although recent studies in some species indicate that during atresia, the follicular and/or nurse cells can be eliminated by different cell death mechanisms, these events were scarcely explored in Triatominae. The aim of this work was to investigate the cell death mechanisms operating during follicular atresia induced by nutritional deprivation in Dipetalogaster maxima, a vector of Chagas' disease. For the study, we performed light, fluorescence and electron microscopy, TUNEL assays, western blot and immunofluorescence. The results showed that, in D. maxima, morphological changes during follicular atresia were established gradually. At early atresia, follicular cells exhibited apoptotic nuclei, DNA fragmentation and the presence of activated caspase-3 proteases. At late follicular atresia, cellular changes were more pronounced, and cytoplasm vacuolization and abundant autophagic vacuoles were clearly observed. At this stage, an intense fluorescence pattern of LC3-II, a marker of autophagy, and ultrastructural features compatible with necrosis were observed. Results showed for the first time in a hematophagous insect the occurrence of apoptosis, autophagy and necrosis operating during follicular atresia. Whereas apoptosis played a major role during the early atresia, autophagy and necrosis seemed to be more relevant at late stages of the process. It was shown that follicular atresia in D. maxima is a complex process, signed by biochemical and cellular changes which correlate with the time elapsed since the instauration of atresia.

Keywords:*Follicular atresia, Cell death, Chagas' disease vectors*

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Physiology

P2

Functional analysis of β-ATP synthase as a lipophorin-binding protein in the midgut of *Panstrongylus megistus* (Hemiptera: Reduviidae)

Leonardo Luis Fruttero[1], Celia Regina Carlini[2], Edilberto Rene Rubiolo[3], Lilian Etelvina Canavoso[4]

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Lipophorin, the main insect lipoprotein, is a "reusable shuttle" that takes up or delivers lipids to tissues without being internalized and degraded. Thus, the process of lipid transfer involves the interaction of lipophorin with specific sites in the plasma membrane of the cells. Despite the relevance of this event in the physiology of the insects, the identification of non-endocytic lipophorin receptors is still lacking. The aim of this work was to characterize the role of the β subunit of the ATP synthase (β-ATPase) as a lipophorin-binding protein in the midgut membrane as well as its involvement in the transfer of lipids. Fifth instar nymphs of Panstrongylus megistus, an important vector of Chagas' disease in South America, were used for the experiments. This hematophagous species can take large blood meals containing a substantial amount of lipids. The results, obtained by ligand blotting followed by tandem mass spectrometry, identified the β-ATPase as a lipophorin-binding protein. The expression of β-ATPase, as measured by RT-PCR and western blot (WB), changed according to the physiological condition of the insects. Subcellular fractionation and WB detected β-ATPase in cellular membranes. The immunofluorescence analysis evidenced a partial co-localization of β-ATPase and lipophorin in the sub-epithelial layer of the midgut. Functional in vivo studies injecting an anti-β-ATPase antibody to block the lipophorin-β-ATPase interaction resulted in an important inhibition of the lipid transfer from lipophorin to the midgut. Taken together, these findings strongly suggest that β-ATPase plays a role as a non-endocytic lipophorin receptor in the midgut of P. megistus.

Keywords:*Lipophorin, β-ATPase, Midgut*

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Physiology

P2

Follicular atresia in the hematophagous *Dipetalogaster maxima* (Hemiptera: Reduviidae): the involvement of acid phosphatase and cathepsin D

Jimena Leyria[1], Silvina Andrea Aguirre[2], Leonardo Luis Fruttero[3], Celia Regina Carlini[4], Edilberto Rene Rubiolo[5], Lilian Etelvina Canavoso[6]

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In insects, unfavourable nutritional conditions induce biochemical and cellular changes in the ovarian tissue such as early degradation of vitellin (Vt), the main yolk protein, and the atresia of several follicles. In some species, the changes accomplished during atresia are elicited to promote the recycling of proteins to sustain younger follicles. In this study, we have explored the biochemical changes that take place during the process of follicular atresia induced by a nutritional deficit in Dipetalogaster maxima, a vector of Chagas' disease. We have emphasized in the roles of two acid hidrolases, cathepsin D (CD) and acid phosphatase (AP). The study included western-blot, the measurement of enzymatic activities, immunofluorescence as well as in vitro assays of Vt proteolysis employing specific inhibitors of CD and AP. Results demonstrated that proteolysis of Vt occurred gradually during the transition from vitellogenesis to follicular atresia. Higher activities of AP and CD were detected at early and late atresia, in comparison to pre-vitellogenesis and vitelogenesis. In vitro assays showed that AP is important in promoting Vt degradation, which in turn was mediated by CD. Altogether, the biochemical changes exhibited during follicular atresia in D. maxima could reflect the ability of the ovarian tissue to either, face another vitellogenic cycle if the nutritional status improve or increase female lifespam. The results also provide a better understanding of the biology of reproduction of Chagas' disease vectors which could be of potential use in vector control strategies.

Keywords:*Chagas'disease vectors, Follicular atresia, Acid hydrolases*

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PS2M150

Physiology

P2

Analysis of the AA-NAT (aralkylamine N-acetyltransferase) expression in different tissues during development of the cotton leaf worm, *Spodoptera littoralis*

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Melatonin has been detected in organisms representing all phyla and it plays crucial role as mediator of the photoperiodic information. However in contradistinction to vertebrates the physiological role and metabolism of melatonin in insects is still poorly understood. Moreover some reports show, that in contrast to the organs of vertebrates, in which melatonin rises during the night, in examined insects tissues melatonin peak occurs during the day. These changes are an effect of the daily activity of the key enzyme in melatonin biosynthesis pathway, the aralkylamine N-acetyltransferase (aaNAT). In several studies it has been also shown that the insect aaNAT has at least two isoforms with different activities dependent on the pH. Here we show temporal patterns of the aaNAT activity in various larval and adult organs of the cotton leaf worm. Our data indicate differences of the diurnal aaNAT activity in all examined organs that is strongly dependent on the pH. Additionally we demonstrate differences between day and night expression patterns of the aaNAT gene. It seems that all tissues of the cotton leaf worm larvae and adults have their own and unique temporal pattern of the aaNAT gene expression and enzyme activity.
Supported by NCN Grant No. N N303 595639 to P.B.

Keywords:*melatonin, aaNAT, rhythm, Spodoptera littoralis*

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PS2M151

Physiology

P2

A genome-wide survey for RNAi genes in rice brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae)

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As an evolutionarily conserved gene-regulatory mechanism, RNA silencing occurs in a wide variety of eukaryotic organisms. In brown planthopper N. lugens, a major rice pest in Asian, available studies indicated that this species is amenable to systemic RNAi by approaches of delivering dsRNA either by microinjection or by feeding. Understanding the RNAi mechanism in this species might help us employ this technique to explore its biological features and control this pest in agriculture. The completed genomic sequence of N. lugens allowed us to comprehensively investigate the repertoire of genes involved in RNAi pathway by comparison with homolog genes from fruit fly D. melanogaster, red flour beetle T. castaneum and nematode C. elegans. Our analysis illustrated that the N. lugens genome encodes all RNAi core components and some genes implicated in systemic RNAi found in T. castaneum. The Brown planthopper retains one Drosha and two Dicer, four dsRNA binding motifs (dsRBM) proteins, five Argonaute proteins, two Snipper-like proteins, a sid-1-like protein and an systemic RNAi related RSD protein (RSD-3), but no RdRP-related protein was found as reported in other insects. A ScanProsite search revealed that some RNAi core components in N. lugens showed distinct features. A phylogenetic analysis of Dicer proteins implicated that N. lugens RNAi pathway is more similar to T. castaneum than D. melanogaster, the latter does not show a robust systemic RNAi response. The brown planthopper N. lugens might be a good model system to study the molecular mechanism of systemic RNAi in insects.

Keywords:*Brown planthopper, systemic RNAi,*

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PS2M152

Physiology

P2

Transcriptomics of bacteriome and spermalege of the bedbug *Cimex lectularius*

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The bedbug Cimex lectularius (Hemiptera: Cimicidae) is an obligate blood-feeding exoparasite of human and other warm-blooded animals. In addition to its medical and hygienic importance, C. lectularius is known for its unique biological traits, which include the obligate nutritional mutualism with a vitamin-provisioning Wolbachia endosymbiont, and the peculiar mating habit called traumatic insemination. For these unique traits, novel insect organs, the bacteriome for endosymbiosis and the spermalege for traumatic insemination, have evolved in the lineage of bedbugs. We constructed cDNA libraries of the bacteriome and the spermalege of C. lectularius, and performed expressed sequence tag (EST) analyses of these peculiar insect organs. In total 4,480 ESTs were compiled as C. lectularius putative nuclear genes, which were assembled into 2,989 unique sequences (USs). The following ESTs and USs were identified from the different organs: 1,151 ESTs and 901 USs from the female whole body; 1,098 ESTs and 879 USs from the female bacteriome; 1145 ESTs and 783 USs from the male bacteriome; and 1,086 ESTs and 920 USs from the female spermalege. Although EST repertories were quite different between the female bacteriome and the male bacteriome, expression levels of antioxidant genes were commonly high in both the bacteriomes. The EST data of the spermalege library revealed a number of candidate genes of C. lectularius that are presumably relevant to its immune and defensive aspects. These EST data will provide a valuable genetic resource for understanding the developmental and evolutionary aspects of the novel insect organs. [Supported by BRAIN]

Keywords:*Endosymbiosis, Traumatic insemination, Symbiotic organ, Expressed sequence tags*

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Physiology

P2

Juvenile hormone titer and JH signaling gene expressions during differentiation of neotenic reproductives in the termite *Reticulitermes speratus*.

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The reproductive castes of termites include primary reproductives, which found a new colony, and neotenic reproductives which take over reproduction when primary reproductives die or are senescing. Neotenic reproductives usually differentiate from several stages of nymphs or workers and retain juvenile characteristics and functional gonads. Therefore, the organ formation of neotenic reproductives must be different from those of alates. However, developmental mechanisms during differentiation of neotenic reproductives are unknown. To clarify these mechanisms, we performed analyses on morphological and physiological changes during differentiation of neotenic reproductives of the termite Reticulitermes speratus. Morphological observations showed that wing-bud and compound-eye developments of the newly emerged neotenic reproductives were suppressed and significant ovarian development did not occur. However, vitellogenin gene expression levels in newly emerged neotenic reproductives were remarkably higher than those in nymphs. Next, we quantified the JH titer of each developmental stage using LC-MS. Our results showed that JH titers of newly emerged and mature neotenic reproductives were significantly higher than those of nymphs. Finally, JH signaling molecule (including Methoprene-tolerant which is a putative JH receptor gene) expression changes were analyzed by real-time quantitative PCR. The results showed that gene expressions in abdomen of neotenic reproductives were significantly higher than those in head and thorax. These results suggest that physiological changes occur soon after molts into neotenic reproductives, and their organ-specific developments are regulated by the organ-specific expression of the JH signaling pathway.

Keywords:*Juvenile hormone, Caste differentiation, Termite*

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PS2M154

Physiology

P2

Molecular developmental mechanisms responsible for soldier-specific morphogenesis in the nasute termite *Nasutitermes takasagoensis*.

Kouhei Toga[1], Kiyoto Maekawa[2]

[1]*University of Toyama, Japan,* [2]*University of Toyama, Japan*

Social insects possess morphologically and behaviorally different castes, even though they have almost the same genetic backgrounds. Although juvenile hormone (JH) plays an important role in determining caste developmental fate, developmental mechanisms leading to morphological modifications are poorly understood. Termite soldier differentiation provides a tool for understanding the developmental mechanisms for morphological modification, because molts into presoldiers (the transitional stage prior to soldiers, possessing the fundamental morphologies of soldiers) can be induced artificially by JH application. Soldiers of the termitid subfamily Nasutitermitinae, one of the most derived termite groups, possess a long and horn-like frontal projection (nasus), an invaginated glandular structure in the head (frontal gland), and regressed mandibles. To investigate whether patterning gene and programmed cell death (PCD) are involved in soldier-specific organ formations of Nasutitermes takasagoensis, we performed RNAi for morphogenetic factors such as Distal-less (Dll) and TUNEL staining for the detection of PCD. RNAi showed that Dll was involved in nasus elongation, but not frontal gland formation. TUNEL staining showed that PCD was involved in mandibular regression. These results suggest that soldier-specific organ formation occurs by modifications of preexisting developmental mechanisms. Based on these results, together with analyses on other factors involved in cell proliferation (e.g. insulin signaling), we will discuss the molecular mechanisms of soldier-specific organ formation in termites.

Keywords:*termite, caste differentiation, organ formation*

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PS2M155

Physiology

P2

Aim-then-shoot type of mechanisms involved in the jumping approach of potato moths to a sex pheromone source

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Male moths track an odor plume of sex attractant pheromone emitted by conspecific females. It is widely accepted that for flying moths, upwind flight is essential for tracking pheromone, and it is achieved during flight by detecting a visual flow, termed optomotor anemotaxis. However, pheromonally excited males of potato moth, Phthorimaea operculella, exhibit a series of intermittent short-straight flights, or jumps, while going upwind. It is hardly conceivable that they navigate themselves by employing the same mechanism as flying moths use. To analyze odor-conditioned anemotaxis of potato moth males, we constructed a flat wind tunnel sandwiched between a pair of glass plates, in which a plane of air flows from one side to the other. A computer-regulated valve system introduces pheromonally-permeated air as pulses into the air flow, thus it forms a series of odor bands separated by gaps of clean air. This enabled to give odor stimuli regularly to an insect regardless of its location in the wind tunnel. Path analyses revealed that males surged upwind when they encountered a pheromone pulse, and released casting with successive jumps when they lost odor. This behavioural pattern seems to be similar to that of flying moths. When the direction of the air flow was switched orthogonally, however, males could adjust course angle accordingly while they were on the ground. It is suggested that instead of optomotor cues, mechanosensory means might be adopted to detect wind direction by 'aim-then-shoot' mechanisms. Here we propose some evidence.

Keywords:*behavior, orientation, moth, pheromone, anemotaxis, wind tunnel, aim-then-shoot*

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PS2M156

Physiology

P2

Diversification of photoreceptors in the compound eye of the birdwing butterfly, *Troides aeacus formosanus*

Pei-Ju Chen[1], En-Cheng Yang[2]

[1]*National Taiwan University, Taiwan,* [2]*National Taiwan University, Taiwan*

Electrophysiological recording in the compound eye of the birdwing butterfly, Troides aeacus formosanus, showed that there are 9 spectral types of photoreceptor with peak sensitivities at 370 nm, 390 nm, 450 nm, 510 nm, 540 nm, 550 nm, 580 nm, 610 nm, and 630 nm. The spectral sensitivities of some photoreceptors do not match to those predicted spectrum of a visual pigment, when fitting the theoretical pigment absorption curves to the recorded spectral sensitivities. The inspection of an intact eye under ultraviolet epi-illumination revealed that some ommatidia exhibit UV-induced fluorescence. The ommatidial heterogeneity is correlated with the red or yellow pigmentation around the rhabdom. Tuning of spectral sensitivities of photoreceptors could be due to the filtering effects by the fluorescing and perirhabdomal pigments in the Troides eye. In addition to the optical filtering effects, negative-going responses were observed, indicating that there are strong antagonistic electrical interactions at the level of photoreceptor. The variety of receptor types caused by the pigments filtering effects and electrical interactions in the Troides eye suggests that the Troides butterfly has a good ability on color discrimination.

Keywords:*color vision, spectral sensitivity, photoreceptor, filtering effect, butterfly*

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Physiology

P2

Functional analysis of epigenetic regulation during embryogenesis of the cricket, *Gryllus bimaculatus*

Yuji Matsuoka[1], Tetsuya Bando[2], Taro Nakamura[3], Taro Mito[4], Sumihare Noji[5]

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Epigenetic regulation is evolutionarily conserved mechanism to maintain correct gene transcriptions for proper developmental processes. In Drosophila embryos, Polycomb group (PcG) genes are one of the epigenetic factors and function in regulation of Hox gene expression after Gap genes expression is diminished. To elucidate the function of epigenetic regulation in development of Gryllus bimaculatus are phylogenetically basal insect, we cloned three PcG genes, Gryllus homolog of Enhancer of zeste (E(z)), Polycomb (Pc) and Suppressor of zeste 12 (Su(z) 12) and performed RNAi. E(z)RNAi embryos were lethal at late stages showing defect in katatrepsis. In the RNAi-ed embryos, the Hox genes, Antennapedia, Ultrabithorax, abdominal-A and Abdominal-B, were ectopically expressed in the whole embryo body, while Sex combs reduced expression was reduced. The antenna, maxilla, labium and three-pair of thoracic legs showed homeotic transformation to the T3 leg, consistently with alteration of Hox gene expressions. Pc and Su(z)12 RNAi-ed embryos also were lethal and defected in katatrepsis, but homeotic transformation of appendages were not occurred. Reduction of trimethylated on hisotneH3 lysine 27 (H3K27me3), which is catalyzed by E(z), was observed in E(z)RNAi embryos but not in Pc and Su(z)12 RNAi-ed embryos. In Gryllus, only E(z)RNAi embryos showed alteration of Hox genes expression pattern as shown in fly and mouse PcG genes mutants, although Drosophila Pc and Su(z)12 mutants showed that. These results suggest that functions of PcG gene constituents, Su(z)12 and Pc, in regulating Hox genes were altered during insect evolution. Furthermore, in the basal Gryllus, PcG genes regulate katatrepsis, different from Drosophila.

Keywords:*Development, Epigenetics*

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PS2M158

Physiology

P2

Expansion of the odorant receptor family and reduction in the gustatory receptor family in the Hessian fly: - Adaptation to a stressful lifestyle?

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The Hessian fly (Diptera, Cecidomyiidae) is a serious pest of wheat in most wheat growing areas in the world. The short adult life (1-2 days) means that flies are forced to accomplish host and mate finding within a restricted timeframe, which puts a high demand on the chemical senses. The insect odorant receptors (ORs) and gustatory receptors (GRs) belong to a large superfamily of chemoreceptors. We annotated 122 ORs and 27 GRs from the Hessian fly genome. Compared to D. melanogaster and A. gambiae, we find drastic expansions of OR lineages in the Hessian fly. The largest expansion contains 71 Hessian fly ORs, and no OR from the other species. Many of these ORs share very high amino acid identity and are located close on chromosomes, indicating recent duplication events. In contrast, we found a considerable reduction in GRs, by at least half, relative to other genome-sequenced Diptera. Hessian fly contains all three CO2 receptors, but has a reduced set of sugar receptors. For both the ORs and GRs, the Hessian fly appears to have lost numerous lineages that might have been present at the base of the Diptera. The expanded OR-, but reduced GR repertoire, might have evolved as a result of specialization on a single host plant and utilization of a long-range sexual pheromone. Possibly when life is short, long-range cues predominate, as there might simply be no time to land on potential hosts or mates and evaluate their quality upon contact.

Keywords:*Odorant receptor, Gustatory receptor, OR, GR, Hessian fly*

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PS2M159

Physiology P2

Changes In the expression of cell cycle markers in moth *Spodoptera littoralis* tissue after exposure to the juvenile hormone analog- Fenoxycarb

Agnieszka Suszczynska[1], Joanna Kotwica-Rolinska[2], Piotr Bebas[3], Marta Anna Polanska[4]

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Physiological processes, including growth and development in insects are controlled by two major hormones: ecdysteroids and juvenile hormones (JHs) which regulate metamorphosis i.e. by acting on cell cycle and apoptosis in most tissues. Juvenile hormone analog (JHA)- fenoxycarb (the carbamate belonging to growth regulators) imitate the action of JH by binding to the same receptors finally causing disruption of insect development and reproduction. In this study we investigated effect of fenoxycarb on cell cycle markers in Egyptian cotton leaf worm Spodoptera littoralis tissues. Animals were treated with different doses of fenoxycarb and next kept in normal conditions for next 24 hours. Then, using the Real-Time quantitative PCR approach we tested the genes expression of cyclin A, cyclin C, cyclin T and Growth Arrest and DNA Damage (Gadd) in gonads, brains and Malpighian tubes, isolated from treated insects. Obtained data indicate that fenoxycarb increases expression of cell cycle markers in gonads, but decreases or does not change cell proliferation markers in the central nervous system and Malpighian tubes. Supported by NCN grant No. N N303 803340 to MAP.

***Keywords**:juvenile hormone analogue, cell cycle regulation, moths*

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PS2M160

Physiology

P2

RNA interference in the termite *Reticulitermes speratus*: a comparison of effect on silencing the hexamerin gene between single 21 nt siRNAs and a long dsRNA.

Shuji Itakura[1], Saho Tatsuuma[2], Toshihiro Teranishi[3], Kaya Hashizume[4], Miyu Kaneda[5]

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We had reported silencing of the hexamerin gene of R. speratus using a single 21 nt small interfering RNA. The single siRNA had been selected in order to avoid off-target effects that could be caused by using long dsRNA or a mixture of siRNAs of various lengths (15 - 25 bp) prepared by digestion of dsRNA. To confirm effectiveness of a single siRNA, three kinds of 21 nt siRNAs and a long dsRNA (585 bp) were prepared from the hexamerin sequence of R. speratus (AB371986). The target sequences for siRNAs (330-348, 2107-2125, 2273-2291 nt) were designed using the siRNA Target Designer program (http://www.promega.com/siRNADesigner/program/), while that for a long dsRNA (635-1219 nt) with low sequence similarity to sequences in the nucleotide databases was selected using BLASTN. The siRNA and dsRNA injection treatments (injection volume 2.3 nl, 3.0 ng) used to silence hexamerin caused moderate suppression of hexamerin gene expression in workers for 3 days after the injection. In 5 days, hexamerin gene expression recovered in workers injected with two kinds of siRNAs and a long dsRNA, while suppression continued in one of siRNA treatments. Hexamerin gene expression in workers injected with three kinds of siRNAs and dsRNA was recovered and rose about twice (a siRNA and a long dsRNA), 8 times (a siRNA), and 26 times (a siRNA) in 7 days after injection. Present study showed that single 21 nt siRNAs and a long dsRNA suppressed equally hexamerin gene expression in workers of R. speratus for three days after injection.

Keywords:*double-stranded RNA, RNA Interference, a single 21 nucleotide siRNA*

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Physiology P2

Molecular cloning and analysis of a novel beta-1,3-glucan recognition protein from the ghost moth, *Thitarodes pui* (Lepidoptera, Hepialidae)

Wenjing Wu[1], Zixuan Sun[2], Min Zhang[3], Chao Song[4], Guren Zhang[5]

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Detection of pathogen surface molecules is the crucial first step of host defense reactions. Beta-1,3-glucan recognition proteins (βGRPs) are soluble pattern recognition proteins that bind to β-1,3-glucans from fungi cell walls and initiate immune responses in insects. A novel βGRP gene was cloned from the ghost moth Thitarodes pui, narrowly distributed at an altitude of 4,100 to 4,650 m in the Mt. Sejila of the Tibetan Plateau. The βGRP was classified into βGRP clade 4 in phylogenetic analysis and designated TpβGRPc. The full-length TpβGRPc cDNA (1,915 bp) has a 1,464 bp open reading frame encoding a protein of 488 amino acid residues including a 21 amino acid residues signal peptide, a 127 bp 5' untranslated region (UTR) and a 324 bp untranslated region in the 3' UTR with a 26 bp polyA tail. The calculated molecular mass of the mature protein is 52,596 Da with an estimated pI of 6.06. Tertiary structure of TpβGRPc contains two distinct regions. The N-terminal domain responsible for β-1,3-glucan binding affinity includes ten putative β-strands. The C-terminal domain shows a classical sandwich-like β-jelly roll fold that is similar to β-1,3-glucanases. In phylogenetic tree, TpβGRPs (TpβGRPa, TpβGRPb and TpβGRPc) formed a distinct and separate clade 4 in Lepidopteran βGRP family, which might ascribe to the local distribution of the homoneurous primitive ghost moth.

Keywords:*Thitarodes pui, β-1,3-glucan recognition protein, tertiary structure, phylogenetic tree*

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PS2M162

Physiology

P2

Influence of pymetrozine on feeding behaviors of four major rice sap-sucking insect pests using electrical penetration graphs

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[1]Zhejiang Academy of Agricultural Science, China, [2]Zhejiang Academy of Agricultural Science, China, [3]Zhejiang Academy of Agricultural Science, China, [4]Zhejiang Academy of Agricultural Science, China, [5]Zhejiang Academy of Agricultural Science, China, [6]USDA-ARS, U.S.A.

Pymetrozine is a selective insecticide with the basic structure of a pyridine azomethine, and it is effective against sap-sucking insects. However, the mechanism of feeding inhibiting of pymetrozine to sap-sucking insects is still unknown. The results showed that pymetrozine primarily functioned as an antifeedant to cause starvation and death to Nilaparvata lugens, rather than neuro-toxicity. Pymeytrozine-treated insects died at a significantly slower speed than starvation-treated insects. Pymetrozine significantly inhibited phloem ingestion, and the inhibition was strongly dose-correlated. The phloem ingestion was totally suppressed when the pymetrozine concentration increased to 400 mg litre-1. Starvation caused by inhibition of phloem ingestion might be a major toxicity mechanism of pymetrozine. However, pymetrozine had no effect on the xylem ingestion. It is likely that pymetrozine-treated insects, could sustain for a while on water and limited nutrients from xylem ingestion before they died. On pymetrozine-free rice plants, Sogatella furcifera and Laodelphax striatellus showed a significantly less activity in phloem phases than N. lugens or Nephotettix cincticeps, while N. cincticeps exhibited relatively more xylem ingestion than N. lugens, S. furcifera and L. striatellus. On the plants treated with 100 mg litre-1 of pymetrozine, all four insect pests showed significant increases in total duration of non-probing and significant decreases in the activities in phloem tissue, and similar feeding behavior during the pathway and xylem phases. This revealed that their feeding behavior was disturbed by pymetrozine and exhibited similar patterns of sharp decline in activity in the phloem tissue and a significant increase the non-probing.

Keywords:*pymetrozine, sap-sucking insects, electrical penetration graph, feeding behavior*

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PS2M163

Physiology

P2

Positional cloning of the chocolate 2 (ch-2) gene controlling newly hatched larvae colour in silkworm, *Bombyx mori*.

Rui Zhang[1], Chengxiang Hou[2], Xinglin Mei[3], Anjiang Tan[4], Anying Xu[5], Yongping Huang[6], Muwang Li[7]

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Abstract: In the silkworm, many mutants exhibiting newly hateched larvae colour have been found. One of the most interesting mutants is ch-2(chocolate, 18-0.0). The body-color of newly hatched larvae in ch-2 homozygous individuals are reddish brown. To better understand the colour formation mechanism of newly hatched larvae in silkworm, we tried to isolate the gene responsible for ch-2 based on positional cloning. Seven polymorphic markers were selected from the SSR linkage maps (Miao et al, 2005). Fifty-Seven pairs of STS primers were designed according to the sequence of nscaf2901 on chromosome 18, and 12 of them had been found to be polymorphic between the parents, k04(ch-2) and p50. Using these primers, ch-2 had been mapped in a region of 300kb on nscaf2901 by using 477 backcross progenies. There are 8 predict genes in this region. They were all expressed as mRNA in embryo. After comparing the cDNA sequences of thes genes between p50 and k04, we found a deletion of 146bp in cuticular protein glycine-rich 6 (CPG6) (BGIBMGA008245) in K04 according to normal P50, we cloned the sequence and obtained a full length cDNA. Based on the cDNA sequence, we designed and synthesize siRNA to interfere the BmCPG6 gene expression in P50 embryo, and we found most of the colour of newly hatched larvae changed to chocolate. Based on these results suggest that a mutation in BmCPG6 gene causes the phenotype of ch-2.

Keywords:*Positional cloning, chocolate 2 (ch-2), cuticular protein glycine -rich 6 (CPG6), Bombyx mori*

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PS2M164

Physiology

P2

Water physiology of vitellogenic follicles in *Bombyx mori*

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Ovarian follicles pass through a growth phase during which the developing oocyte endocytoses the vitellogenin and other yolk proteins for storage in its cytoplasm. It has long been recognized that an oocyte increases its size by water absorption during this process. We have raised a question whether an aquaporin (AQP: water channel) is necessary for oocyte swelling between onset and termination of oocyte maturation. By the immunoblotting test with three specific antibodies directed to each Bombyx AQP, we found the strong bands around 110-kDa, which appears to be the tetrameric nature of AQP-Bom3 [PRIP] from the developing ovaries (vitellogenic follicles). When sections of vitellogenic follicles from the female pupa (Day 6 ovary) were stained with anti-AQP antibody for Bombyx PRIP, the stain was localized at the periphery of an oocyte and not at any regions of follicular epithelium as well as the nurse cells. This AQP may have a role admirably suited to the egg growth. The other water-specific AQP (AQP-Bom1 [DRIP]) appeared at a certain type of yolk granules distributed in the cortex of an oocyte on the post-vitellogenic phase (Day 9-10 ovary). The significance of AQP-Bom1 in some yolk granules was unclear, but these data suggested that two water-specific AQPs function in developing ovaries of B. mori spatially and temporally.

Keywords:*water channel, aquaporin, Pyrocoelia rufa integral protein (PRIP), yolk granules, oogenesis, Bombyx mori*

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Physiology

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Embryonic development of Zoraptera and its phylogenetic implication

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Zoraptera is one of the least diverse and most poorly understood insects. Until now more than 10 different phylogenetic positions have been proposed. The term "Zoraptera problem" is coined to highlight the highly controversial status of the most enigmatic insect, after the "Strepsiptera problem". Recently, detailed morphological studies on Zoraptera have emerged, but information on its embryonic development is still lacking. In this background, we have started the embryological study of Zoraptera, using Zorotypus caudelli Karny. Embryonic development of Z. caudelli is characterized by the following features: 1) embryo anlagen with a pair of higher cell density areas, 2) embryogenesis of short germ band type, 3) embryo's elongation along the ventral surface, 4) parallel migration of embryo into the yolk after its full elongation on the egg surface, 5) revolution with embryo's anteroposterior axis reversed, and 6) egg tooth extraordinarily elongated on the head capsule. Zoraptera agrees with Polyneoptera, sharing the features 1), 3) and 4) with polyneopteran members, whereas it differs in embryogenesis from Paraneoptera, in which the embryo gradually immerses into yolk from its rear, elongating posterior, to take its position deep in the yolk. This may suggest that Zoraptera is a member of Polyneoptera, being not of Paraneoptera. Our recent studies on Zoraptera revealed close resemblances in egg structures (Mashimo et al., 2011) and male reproductive systems (Dallai et al., 2011) between Zoraptera and Eukinolabia (= Phasmatodea + Embioptera). The feature 6) "long egg tooth" as found in Zoraptera had been only reported in Embioptera (Jintsu, 2010)

Keywords:*Zoraptera, Polyneoptera, Blastokinesis, Egg tooth, Egg structure, Comparative embryology*

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Physiology

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Effect of entomopoxvirus infection on growth and metamorphosis of *Mythimna separata* larvae

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Insect viruses are among the most important parasitic natural enemies of lepidopteran insects. Virus-infected larvae show developmental defects including a prolonged larval period and a failure to pupate, but an overview of the mechanisms by which insect viruses regulate host development has not yet been established. Larvae infected with members of the Baculoviridae (double-stranded circular DNA viruses) fail to pupate because host ecdysteroids are inactivated by ecdysteroid UDP-glucosyltransferase (EGT), an enzyme that is encoded by most baculovirus genomes. However, entomopoxviruses (EPVs), which belong to the Poxviridae and possess a double-stranded linear DNA genome, also prolong the larval period and prevent host pupation, although no EPV has yet been found to encode EGT in its genome. Thus, EPVs are likely to have a different mechanism from baculoviruses to regulate host metamorphosis. In this study, the endocrinological regulation of a lepidopteran EPV, Mythimna separata EPV (MyseEPV), was examined. When M. separata larvae were inoculated at 4th instar with 104 MyseEPV occlusion bodies/larva, pupation was prevented and the insects died in the larval stage after prolongation of the larval period by an average of 6.95 days. The hemolymph of healthy and EPV-infected larvae was collected and the titers of juvenile hormone (JH) and ecdysteroids were measured. LC-MS measurement revealed that JH titers in MyseEPV-infected larvae in the final instar were higher than those in healthy larvae. Ecdysteroid titer was measured using anti-ecdysteroid serum by radioimmunoassay. The ecdysteroid titer in final-instar MyseEPV-infected larvae was lower than that in healthy larvae.

Keywords:*Mythimna separata, Entomopoxvirus, ecdysteroid, juvenile hormone*

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Physiology P2

Identification of peptidyl factors modulating feeding behavior of the silkworm, *Bombyx mori*

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Phytophagous insects including a lepidopteran species such as the silkworm, Bombyx mori, exhibit regularly occurring feeding behavior. To identify factors involved in the regulation of insect feeding behavior, we established a bioassay to evaluate the activity of feeding initiation by measuring latency to the first bite after sample injection in starved Bombyx larvae. Feeding inhibitory activity was observed in the crude extracts of all examined tissues from Bombyx larvae. We then purified feeding-related factors from the extract of the Bombyx larval midgut because of its strongest activity. Four steps of purification by reversed-phase HPLC allowed us to isolate two peptidyl factors. Amino acid sequence analyses revealed that one was allatotropin composed of thirteen amino acid residues, and the other was a novel peptide composed of seventeen amino acid residues, designated GSRYa after the C-terminal sequence. We also examined the feeding activities of several synthetic peptides which have been identified comprehensively in silico using this bioassay. The results showed that five more peptidyl factors were related to feeding behavior in B. mori (allatostatin, myosuppressin, calcitonin-like diuretic hormone 31, short neuropeptide F, and tachykinin). In the present study, we also discuss how these peptidyl factors can modulate the feeding behavior in insects.

Keywords:*Bombyx mori, feeding behavior, peptide*

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Physiology P2

Ovarian development in females of *Triatoma sordida* Stal (Hemiptera: Reduviidae: Triatominae) under fasting conditions.

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Triatoma sordida is a secondary vector of Trypanosoma cruzi - causative agent of Chagas disease - considered a species of epidemiological importance. It is often found in the peridomestic habitat and frequently invade and colonize human dwellings. The study of aspects of the reproductive capacity of these species is relevant for the relationship with colonization of new habitat. The object of the present work was to characterize through morphometric and histological studies the ovarian development in females of T. sordida in absence of food and mating, which were killed at different days post-ecdysis. Morphological changes of the ovaries were estimated by morphometric analysis of basal follicle of each ovariole and oocytes number for different phases of oogenesis. For the histological study the ovaries were embedded in paraffin, processed routinely for hematoxylin?eosin staining. The results indicate that T. sordida presents a quantitative pattern appropriate to identify the three phases of oogenesis. The mean length of basal follicle varied between different phases (0.358±0.096 mm pre-vitellogenesis, 0.638±0.331 mm, vitellogenesis and 1.929±0.136 mm choriogenesis). At the 2nd day post-ecdysis, 100% of the females showed pre-vitellogenic oocytes and 50% showed vitellogenic oocytes at the 4th day. The choriogenic and ovulation processes began at 8th day post-ecdysis. The morphological changes observed in the basal follicles were consistent with the results of histological analysis. These results would indicate that, at 6 days post-ecdysis, females of T. sordida without feeding have eggs ready to be oviposited.

Keywords:*Triatoma, Oocytes, Vitellogenesis*

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Physiology

P2

Effect of the juvenile hormone on resistance against pathogen differs between sexes

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Juvenile hormone is suggested to be a potential mediator in trade off between sexual signaling and immune defence and also trade off between mating and immunity in insects. Studies in a variety of insect taxons have found that juvenile hormone corrupts humoral and cellular immunity. However, studies using real parasites and pathogens are lacking so far. In this study, we tested if the administration of the juvenile hormone have effect on the resistance against an entomopathogenic fungi, Metharizium anisopliae by usingn the meal worm beetle, Tenebrio molitor, as a model species. In previous studies with T. molitor juvenile hormone has been found to reduce a major humoral immune effector-system (phenoloxidase) in both sexes and to reduce the encapsulation response in males. However, we found that juvenile hormone administration prolonged survival time after infection , but reduced survival time in females. Thus, juvenile hormone treatment enhances resistance against the entomopathogenic fungi in males but reduced in females. Results of this study show that the effect of the juvenile hormone on insect immunity may be more complicated than it has been previously thought. We also suggest, that there might be a trade off between specific and non-specific immunity, since in males juvenile hormone enhance specific immunity but corrupts non-specific immunity. Our study highlights the importance of using real parasites and pathogens in immunoecological studies.

Keywords:*ecological immunology*

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Physiology P2

Biological activities of juvenile hormone skipped bisepoxide (JHSB3) in the stink bug, *Plautia stali*

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Juvenile hormone III skipped bisepoxide (JHSB3), methyl (2R,3S,10R)-2,3;10,11-bisepoxyfarnesoate was recently determined as a novel juvenile hormone (JH) in the stink bug, Plautia stali. In the previous study, the juvenilizing activity of JHSB3 was tested only in a limited dose range. To further confirm the biological function of JHSB3 in this insect, not only juvenilizing activity in a wider dose range, but also reproduction-stimulating and diapause-terminating activities were examined. Topical application of JHSB3 to last instar nymphs inhibited their metamorphosis in a dose-dependent fashion. In allatectomized and diapausing adults, JHSB3 application exerted stimulatory effects on the development of the ovaries and ectadenia in females and males, respectively. In the previous study the in vitro product of the corpora allata was used as a biological sample, but its presence in the hemolymph has not been examined yet. The present study asked whether JHSB3 was detected from the hemolymph. Gas chromatography-mass spectrometry analysis showed that JHSB3 content in the hemolymph samples from reproductively active females was relatively high while its content in the hemolymph from diapause females was below the detection limit. These results demonstrated that JHSB3 has biological function as a JH in P. stali. Topical application of stereoisomers of JHSB3 and 10R-JH III also indicated that compounds with the 2R,3S-configuration were more potent than those with the 2S,3R-configuration and 2,3-double bond.

Keywords:*juvenile horomone, stink bug, Pentatomidae, Hemiptera, metamorhposis, reproduction*

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Physiology

P2

Study on microbiota diversity and cellulolytic bacterial community in the hindgut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae)

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Gut microbiota has diverse ecological and evolutionary effects on their hosts. However, the ways in which it responds to environmental heterogeneity remain poorly understood. To this end, we surveyed intestinal microbiota of Holotrichia parallela larvae from different geographic regions. Firmicutes and Proteobacteria were the major phyla, and bacteria belonging to Ruminococcaceae, Lachnospiraceae, Enterobacteriaceae, Desulfovibrionaceae and Rhodocyclaceae families were commonly found in all natural populations. However, bacterial diversity (Chao1 and Shannon indices) and community structure varied across host populations, and the observed variation can be explained by soil pH, organic carbon and total nitrogen, and the climate factors (e.g., mean annual temperature) of the locations where the populations were sampled. Our results suggest that the gut's bacterial community changes in response to environmental heterogeneity, possibly to meet the host's ecological needs or physiological demands. Furthermore, to specifically evaluated the nutritional contributions of gut microbiota to scarabs , we isolated and identified cellulose-degrading bacteria from the gut of H. parallela. Our results showed that the cellulolytic bacterial community is dominated by the Proteobacteria (70.05%), followed by the Actinobacteria (24.15%), the Firmicutes (4.35%), and the Bacteroidetes (1.45%). At the genus level, Pseudomonas, Ochrobactrum, Rhizobium, Cellulosimicrobium, and Microbacterium were the predominant groups. However, some bacterial isolates are reported to be cellulolytic for the first time in this study, indicating that the scarab gut is an attractive source for the study of novel cellulolytic microorganisms and enzymes useful for cellulose degradation.

Keywords:*Holotrichia parallela,hindgut,microbiota diversity,cellulolytic bacterial*

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Physiology

P2

Molecular diversity and function on host of the intestine bacteria of *Bactrocera dorsalis* adults

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In this paper, the 16S rDNA cloned libraries and DGGE fingerprinting was used to investigate bacterial diversity in the intestinal tract of lab-reared, lab sterile sugar-reared and field-collected populations of Bactrocera dorsalis adult. The results revealed that Gammaproteobacteria was dominant in the all samples (73.0%?98.3%). Actinobacteria and Firmicutes were judged to be major components of a given library since they constituted 10% or more of the total clones of such library. The Flavobacteria, Deltaproteobacteria, Bacteroidetes, and Alphaproteobacteria were observed in small proportions. LIBSHUFF analysis showed that the bacterial communities of B. dorsalis from the three populations were significantly different from each other ($P < 0.0085$). The whole beer、filtered and autoclaved supernatants of fermentation cultures of the cultured gut bacteria had attractiveness to B. dorsalis adults. Autoclaved supernatants were significantly more attractive than the whole beer or filtered supernatants. Six isolates, which autoclaved supernatants were most attractive, were selected to attract to B. dorsalis adults in field. Results showed that LRC38 was more attractive than others. The effect of the dominant Enterobacteriaceae community and the minor Bacillus community on B. dorsalis was assessed. The results showed that feeding on Bacillus had a negative effect on longevity while feeding on the Enterobacteriaceae mixture had a beneficial effect. Compared to flies fed on sugar alone, flies fed on Bacillus died at a significantly faster rate and their average longevity was significantly reduced. Conversely, flies fed on Enterobacteriaceae died more gradually and lived significantly longer.

Keywords:*Bactrocera dorsalis,DGGE,intestine bacteria,molecular diversity*

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Physiology

P2

Comparative analysis of major transcripts from the salivary glands of two insect-feeding hemipteran bugs, *Agriosphodrus dohrni* and Orius strigicollis

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There are a number of hemipteran bugs feeding on insects, and some of them are utilized as biological control agents. Although their saliva must contain specialized enzymes and toxins for feeding their preys effectively, the information on these components is limited. Two insect-feeding bugs, an assassin bug Agriosphodrus Dohrn (Ad, Reduviidae) and a flower bug Orius strigicollis (Os, Anthocoridae), are less related phylogenetically and have distinctive prey preferences (lepidopteran larvae, spiders and millipedes in Ad, and thrips and spider mites in Os, respectively), posing a question whether their saliva components are considerably different or not. To answer this, we have constructed cDNA libraries from their salivary glands and performed comparative analysis on the major transcripts. Randomly selected 470 clones for each library were sequenced and classified into 180 and 169 unique transcripts for Ad and Os, respectively. These transcripts of Ad (Os) were further subdivided into 41 (41) housekeeping gene homologs, 27 (12) digestive enzyme and protease gene homologs, 27 (12) other gene homologs, and 85 (104) transcripts with unknown function (including those with no homolog). Comparison of Ad and Os transcripts revealed that homologs of hemolysin and trialysin-like pore-forming proteins of mammalian blood-sucking bug, Triatoma infestans (Reduviidae), were abundantly transcribed in Ad but not in Os, while a homolog of ADO1 toxin of Ad was identified in Os, suggesting that there are a few conserved salivary proteins among insect-feeding bugs, although the other components varied among species due to phylogenetic constraints.

Keywords:*salivary gland, transcriptome, Agriosphodrus Dohrni, Orius strigicollis*

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Physiology

P2

In vitro assay using cultured insect cells expressing mosquito acetylcholinesterase mutants predicts development of resistance to novel insecticides by single point mutations at a target amino acid

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Acetylcholinesterases (AChEs) play an essential role in neurotransmission at cholinergic sysnapses in animals, and possess a catalytic serine residue, which is a target of organophosphorous and carbamate insecticides. Excess use of these insecticides caused poisoning in humans and other vertebrates, and generated resistant insects. Recently, as effective and safer insecticides, small molecules, which target a cysteine residue found at the AChE active site of many insects but not in mammals, have been proposed (Pang et al., 2009). To predict whether the resistance to the novel insecticides could be developed by single point mutations at the target cysteine residue, we have constructed a series of mutated cDNAs of Culex tritaeniorhychs AChEII, a paralog of Drosophila melanogaster AChE, and their enzymatic activities by transient expression in Ke17 cells derived from Bombyx mori embryos were compared. Although all of the mutants except for a nonsense mutant were produced at quantitatively and qualitatively similar levels with the wild-type AChEII (control), their activities varied and were lower than the control. Among them, three mutants to glycine, phenylalanine and serine residues by single nucleotide substitutions showed activities more than 30% of the control respectively, and the activity of a mutant to leucine by a two-nucleotide substitution was almost comparable to the control. These results by in vitro assay rationalize the prediction that resistance to the novel insecticides targeting the cysteine residue would be developed by single point mutations at the target site immediately after excess use.

Keywords:*acetylcholinesterase, insecticide, Culex tritaeniorhychs, Bombyx mori, insect cell*

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Physiology

P2

The circatidal rhythm of the mangrove cricket persists after removal of the optic lobe.

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It has long been controversial whether the circatidal rhythm is a tidally adapted circadian rhythm and generated by a similar mechanism to the circadian clock. The mangrove cricket, Apteronemobius asahinai, inhabits the intertidal zone and synchronizes their activity to tidal flooding and ebbing cycles. Under constant conditions, this cricket shows two rhythms simultaneously in its locomotor activity. One is a circatidal rhythm alternating active and inactive phases and the other is a circadian rhythm modifying the activity intensity in the circatidal active phase. Because these circatidal and circadian rhythms entrain to periodic inundation and light-dark cycles, respectively, the activity rhythm is controlled by two independent clocks. RNA interference of the circadian clock gene period disrupts only the circadian rhythm but not the circatidal rhythm. This fact suggests a difference in molecular mechanisms between the circatidal and circadian clocks. In the present study, we examined the neural mechanisms of these two clocks. The optic lobe was shown to be the locus of the circadian clock in other crickets, but the locus of the circatidal clock is still unknown. To clarify whether the same neurons generate the circatidal and circadian rhythms, we observed the locomotor activity after surgical removal of the optic lobe. After bilateral removal of the optic lobes, most crickets showed the circatidal rhythm, but the circadian rhythm was not observed. In A. asahinai, therefore, the circadian clock is located in the optic lobe as in other crickets, but the circatidal clock exists out of the optic lobe.

Keywords:*circatidal rhythm, Apteronemobius asahinai, optic lobe*

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Physiology

P2

Glutathione S-transferase levels in bed bugs in Thailand

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Bed bug is a blood sucking insect found in several countries including Thailand. Eradication of this insect from household is difficult due to its behavior and insecticide resistance which can be indicated by an increase in such enzymes as acetylcholinesterase, esterase, glutathione S-transferase (GST), and monooxygenase. The current study aims to investigate the GST levels in bed bugs collected from various locations in Thailand by means of a GST Colorimetric Activity Assay Kit. There were 78 samples of Bangkok strain, 49 samples of Chonburi strain, 43 samples of Phuket strain, 19 samples of Krabi strain, and 8 samples of Chiangmai strain of Cimex hemipterus and 21 samples of Chiangmai strain of Cimex lectularius. There were two subgroups of Bangkok strain: 37 samples of non-insecticide exposed and 41 samples of insecticide exposed bed bugs. The average GST levels were 0.071 and 0.019 µmol/min/mg protein for non-insecticide exposed and insecticide exposed subgroup, respectively. The average GST levels were 0.011, 0.024, 0.017, and 0.010 µmol/min/mg protein for Chonburi, Phuket, Krabi, and Chiangmai strain of Cimex hemipterus, respectively. The average GST level of Chiangmai strain of Cimex lectularius was 0.007 µmol/min/mg protein. In addition, GST levels of insecticide resistance Chiba (Japan) and insecticide susceptible Tokyo (Japan) strain of Cimex lectularius were tested. The average GST levels were 0.018 and 0.030 µmol/min/mg protein for Chiba and Tokyo strain of Cimex lectularius, respectively. These data revealed that GST level should not be used as a single indicator of insecticide resistance status in bed bugs in Thailand.

Keywords:*Glutathione S-transferase, insecticide resistance, bed bug, Thailand*

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Physiology

P2

Role of yeast-like endosymbiotes (YLS) of brown planthopper, *Nilaparvata lugens* (Stal) in the development of resistance its host to imidacloprid, neonicotinoid insecticide

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The brown planthopper (BPH), Nilaparvata lugens, is one of major insect pests of rice production in Asian county. In China, chemical control is still main way for suppressing BPH populations, one of main causes of BPH frequency-outbreak is high resistance of BPH to imidacloprid, neonicotinoid insecticide. So delay and management of BPH resistance to the insecticide will be an urgent and important topic to be solved in rice production. The experiment found some differences in population diversity of YLS in imidacloprid-resistant and -susceptible BPH populations. In which, resistant and susceptible BPH had four and three kinds YLS, respectively. YLS numbers was related to its host activities of detoxification enzymes and resistance level. Activities of carboxylesterase (CarE), mixed function oxidase (MFO), superoxide dismutase (SOD), peroxidase (POD), uricase and lipase in imidacloprid-resistant YLS strain were significantly higher than those in imidacloprid-susceptible strain, while activity of glutathione-s-transferase (GST), catalase (CAT) had no obvious difference between two resistant strains. Under 24-32℃ temperature, the normal degradation half-life of imidacloprid at concentration of 10 mg/L was 48.8-61.3d, the half-life of imidacloprid-susceptible and resistant strains to imidacloprid was 43.6-60.3d and 32.0-39.4d, respectively. This indicated imidacloprid-susceptible strains had no degradation ability, and imidacloprid-resistant strains had some degradation capacity. We further found that temperature of environment, concentration of imidacloprid, resistant level of strains affected significantly degradation capacity of symbiotic strain to imidacloprid. One gene relative with imidacloprid-detoxification in YLS was cloned. Those results will provide the theoretical foundation for reasonable explanation of resistant mechanism of BPH to neonicotinoid.

Keywords:*Nilaparvata lugens, yeast-like symbiotie, imidacloprid, enzyme activity, degradation, PCR-DGGE, gene*

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Physiology

P2

Involvement of a circadian system in the circannual rhythm in the varied carpet beetle *Anthrenus verbasci*

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The varied carpet beetle, Anthrenus verbasci, regulates the timing of pupation by a circannual rhythm. The circadian clock controls various physiological functions in most organisms, but its involvement in circannual rhythms has been controversial. We examined whether a circadian system is involved in the circannual rhythm in A. verbasci. Larval duration under light-dark cycles of 20-26 h was not proportional to the period of the cycles (T). Moreover, there was no significant correlation between the pupation time and the circadian period in adult locomotor activity under constant light. Entrainment of this circannual rhythm to annual cycles is achieved by phase resetting in response to photoperiodic changes. To examine whether a circadian system is involved in the phase resetting of the circannual rhythm, we exposed larvae to light-dark cycles with a short photophase followed by a variable scotophase (the Nanda-Hamner protocol). When T was a multiple of 24 h, i.e., 24, 48, or 72 h, short-day effects were clearer than when T was far from a multiple of 24 h, i.e., 36 or 60 h. Exposure to light-dark cycles of T = 36 h had effects similar to those by exposure to long-day cycles of T = 24 h. We conclude, therefore, that the mechanism generating the circannual rhythm is independent of the circadian clock but a circadian system is involved in photoperiodic time measurement for phase resetting in the circannual rhythm of A. verbasci.

Keywords:*Anthrenus verbasci, circadian rhythm, circannual rhythm, Nanda-Hamner protocol, photoperiod.*

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PS2M179

Physiology

P2

GST underlying the rhythmic resistance against permethrin in the German cockroach

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Even though numerous studies have confirmed the daily variation of susceptibility to xenobiotic compounds in animals, there are a few reports studying molecular mechanisms underlying the daily rhythm of insecticide resistance in insects. In the present study, we investigated the daily susceptibility rhythm to permethrin and the expression level of the delta class glutathione S-transferase (BgGSTD1) gene in Blattella germanica. Male cockroaches were exposed to the same concentration of permethrin at different time points in a light-dark cycle, and results showed that the highest resistance occurred during night time. Furthermore, the circadian control of permethrin resistance can be demonstrated by the fact that the highest resistance took place at subjective night under constant darkness. The mRNA expression of the BgGSTD1 gene in the fat body of B. germanica peaked in the early day time, whereas the enzyme activity of cytosolic GSTs showed no daily fluctuations. RNA interference (RNAi) was employed to study the role of BgGSTD1 in the circadian rhythm of permethrin resistance in B. germanica. Both BgGSTD1 mRNA level and cytosolic GSTs activity were significantly decreased by RNAi. Notably, the RNAi-mediated silencing effect appeared to cause greater reductions in the cytosolic GSTs enzyme activity at night than in the day time. In addition, the survival rates of B. germanica with silenced BgGSTD1 were significantly decreased at night but not in the day time when the cockroaches were exposed to permethrin. These results have demonstrated that BgGSTD1 plays an important role in circadian control of permethrin resistance in B. germanica.

Keywords:*permethrin, RNAi, circadian rhythm, GST, Blattella germanica*

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PS2M180

Physiology P2

DNA methylation during the caste determination in the European honeybees, Apis mellifera

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Differentiation between queen and worker in honeybees is determined by larval nutrition intake. Female larvae up to 3 days after hatching can grow into either queen or worker, and the fate of larva is irreversibly-determined on the 4th day after hatching. To clarify the epigenetic control of caste determination, genome-wide DNA methylation pattern were compared between queens and workers on the 4th day after hatching by next generation sequencing technology. We identified 1,717 CpG sites that showed significant difference in methylation frequency between queens and workers. Interestingly, transformer-2 (tra-2), a sex-determining gene, contained three differently methylated CpG sites. This observation implied that tra-2 is involved in caste differentiation as well as in sex determination. To further examine this possibility, we investigated the methylation pattern of tra-2 in 3- to 5-day old workers and queens by bisulfite sequencing. We report two main results: (1) methylation pattern in larvae drastically changes during developmental stage in both workers and queens, (2) the patterns of the changing are greatly different between castes.

Keywords:*DNA methylation, Honeybee, Apis mellifera, Transformer-2*

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Physiology

P2

Identification of sex pheromone components in *Trissolcus brochymenae* females

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Long- and short-range sex pheromones appear to play a crucial role in the mate finding and courtship behavior of most parasitic Hymenoptera. Yet these parasitoids have been rarely investigated and only a few pheromones have been identified. Recent studies have shown that sexual communication in Trissolcus brochymenae (Hymenoptera: Platygastridae), a quasi-gregarious egg parasitoid of the harlequin bug, Murgantia histrionica (Heteroptera: Pentatomidae), starts before contact between males and females and is mediated by chemical compounds produced by virgin females, which triggers the courtship behavior in males. In this study, the pheromone components involved in the short-range recognition of T. brochymenae females by males were investigated using electrophysiological and behavioral methods. Female body extracts were analyzed through EAG and GC-EAD and the active compounds were identified through GC-MS. Then the behavioural responses of virgin males to the GC-EAD active compounds were evaluated in closed arena bioassays. Two electrophysiologically and behaviorally active compounds were identified as sex pheromone components, tetradecyl acetate and (Z)-11-hexadecen-1-yl acetate. Both compounds triggered intense male antennation and mounting when applied on solvent washed female cadavers. Dose-response tests showed different curves for the two compounds. This is the first study on the identification of sexual pheromones in Platygastridae. The difficulties of sex pheromone investigations on such minute egg parasitoids and the possible exploitation of these new findings in IPM are discussed.

Keywords:*Platygastridae, egg parasitoid, electrophysiology, tetradecyl acetate, (Z)-11-hexadecen-1-yl acetate, courtship behavior*

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PS2M182

Physiology

P2

The neuropeptide allatostatin A and its receptor DAR-2 regulate metabolism in *Drosophila*

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Knowledge about the neuroendocrine circuits controlling metabolism is important in the struggle to overcome the challenges of diabetes and cardiovascular diseases, and other health problems related to metabolism and food intake. Several elements of metabolic homeostasis are conserved from flies to mammals. The fruit fly Drosophila melanogaster is a powerful genetically tractable model-organism. We are studying a conserved neuroendocrine circuit involving hormones related to glucagon and insulin in mammals, to identify the components and understand their role in the regulation of metabolism. We assume that a key upstream regulator of the circuit is the neuropeptide Allatostatin A (AstA), known to influence feeding behavior. Our data indicate that one of the AstA receptors DAR2 is expressed in three sets of peptidergic neurons, the insulin-producing cells (IPCs), in the adipokinetic hormone (AKH) producing cells of the corpus cardiacum (CC) and in the Corazonin (CRZ)-producing neurons. These hormones are involved in the maintenance of sugar homeostasis and regulation of triglyceride deposits. The AstA neurons directly contact both the CRZ neurons and the IPCs. Furthermore, starvation assays and expression patterns of key metabolic genes, following RNAi mediated knock down of AlstR2 in the CC and IPCs, indicate that AstA is involved in the regulation of AKH, and in insulin release. Taken together, the results indicate that AstA is a regulator of AKH, CRZ and Insulin and that this neuroendocrine system might coordinate feeding and metabolism.

Keywords:*metabolism*

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Physiology

P2

Investigation of lectins as competitors of virus in aphids and potentiality in viral disease transmission

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Awareness of the danger of the excessive use of pesticides to human health and the environment has led to some scientific research to develop alternative strategies for pest management. All these investigations aim to reduce the massive use of toxic chemicals. In order to have efficient control of viral diseases by reducing the aphid vector dispersion virus transmission efficiency, some interesting glycoproteins such as lectins were found to be potential competitors to virus. Indeed, these proteins present large affinity to receptors located from the sucking mouth parts to the hindgut of the vectory aphids. In this study, the effects of three lectins on the transmission of three non-persistant viruses were tested in laboratory on Myzus persicae via the use of artificial diets. The glycoproteins used were Canavalia ensiformis agglutinin (ConA), Galanthus nivalis agglutinin (GNA) and wheat germ agglutinin (WGA) and the viruses studied were PVY (Potato virus Y), CMV (Cucumber Mosaic Virus) and TuMV (Turnip Mosaic Virus). An interference between GNA and all viruses was observed. In addition, a competition between WGA and two viruses i.e. CMV and TuMV was highlighted. In consequence, the two latter lectins were considered as promising proteins to reduce the transmission of these viruses. Strategies based on pre-feeding aphids with lectins before potential virus transmission and application in fields were discussed in terms of sustainable and efficient reduction of viral disease in crops.

Keywords:*Aphid-virus interaction, lectins*

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Physiology

P2

Effect of reduced water supply on aphid physiology : a proteomic approach on peach-aphid interaction

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Whether the effect of water lacking was mainly studied on plant responses, very few investigations were developped on the herbivore responses to water deficiency in host plant. Here, a proteomic approach related to clones of the green peach aphid Myzus persicae which was exposed to normal and water deficient irrigation was studied. After 48 hours, as compared to aphids fed on control plants (well-watered), the aphids fed under water-deficit exhibited several proteins up-regulated. Most of the proteins exhibiting changes were involved in energy metabolism (as Regulator of G-protein signaling 7-like and RNA 3' terminal phosphate cyclase), or were associated to cytoskeleton functionality (as actin related protein 1 and F-actin capping protein subunit beta). One of these proteins was significantly down-regulated, the mitochondrial-processing peptidase, which is associated with aphid response to toxicity. Moreover, some proteins from the Buchenra aphidicola were found to be deregulated. Presence of only primary symbiont was assessed by PCR approach. In a parallel experiment using similar treatments as in the proteomic study, the population rate of growth (PGR) of aphids were determined. These findings suggested that plant under water deficit suffers of physiological changes which in turn elicit (or "transferred to") a significant proteomic changes on aphids fed on them.

Keywords:*water stress, aphid, proteomics*

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Physiology P2

Termites artificially-fed on unusual diet and resulting enzymatic switches

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Wood-feeding termites as Reticulitermes santonensis generally feed on cellulose, hemicelluloses and lignin. However, these opportunistic insects are also able to degrade other carbohydrates, such as starch. The production of putative endogenous α -amylase has been previously shown in R. flavipes, as the disappearance of the major symbiotic flagellates from the hindgut. Here, we compared enzymatic activities (CMCase, MCCase, xylanase, amylase, α- and β-glucosidase) between different fractions of the digestive tract of starch-, cellulose-, and wood-fed termites. Main compounds of the artificial diets, namely starch or MCC, resulted in differential enzymatic activity. Even the substitution of wood by artificial diets itself seemed to induce changes in enzymatic activities, regardless of the main substrate in the diet, as we observed strong midgut α-glucosidase activity only for artificially-fed termites. Preliminary assays to isolate and characterize enzymes were performed using proteomic methods.

Keywords:*enzymatic activity, lower termites, symbionts, starch, cellulose*

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Physiology

P2

Cloning and expression of a membrane form guanylyl cyclase, BdmGC-2, in the oriental fruit fly, *Bactrocera dorsalis*

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Guanylyl cyclases (GCs), an important group of enzymes, catalyze formation of cGMP for intracellular signal transduction in both mammals and insects. In our current study, a cDNA of membrane form GC has been isolated from the oriental fruit fly, Bactrocera dorsalis (Hendel), designed BdmGC-2. The BdmGC-2 cDNA, with size of 5,750 bp, contains a 4,524-nucleotide open reading frame encoding a protein of 1,508 amino acids. BdmGC-2 contains four functional domains, i.e. extracellular ligand binding domain, transmembrane domain, kinase-like domain and cyclase catalytic domain, which is similar to all known receptor GCs. BdmGC-2 was expressed in all developmental stages of B. dorsalis. Real-time PCR assay revealed that during the pupal development BdmGC-2 expression was gradually reduced in the early stages, yet it was increased again after six days. Furthermore, BdmGC-2 was constitutively expressed in the various tissues of both male and female adults, mainly in the brain, testis and Malpighian tubules. Among them, the Malpighian tubules have the highest amount of BdmGC-2 expression; moreover, whole-mount in situ hybridization has further confirmed the expression of BdmGC-2 in the Malpighian tubules.

Keywords:*guanylyl cyclase, Malpighian tubule, Bactrocera dorsalis*

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Physiology

P2

Hemolymph melanization in the silkmoth *Bombyx mori* requires the formation of a high molecular mass complex that metabolizes tyrosine

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A key immune response exhibited by arthropods is the melanization of hemolymph. The enzyme phenoloxidase (PO) is thought to assume the same metabolic role in melanin formation as a class of enzymes called tyrosinases, which oxidize tyrosine (Tyr) and o-diphenols to their corresponding quinones. However, purified POs only poorly metabolize Tyr, and the actual melanization substrate has not been unequivocally identified. We have found that, unlike purified PO, Tyr is rapidly converted to melanin in substrate-free plasma (SFP) from B. mori larvae. HPLC separations of B. mori plasma showed only a single fraction containing a melanin-producing substrate, which was identified as Tyr. Tyr rapidly disappeared in melanizing B. mori plasma, while phenylthiourea, an inhibitor of melanization, blocked its metabolism. To determine what was utilizing tyrosine, plasma from B. mori was separated by gel filtration chromatography. Tyr-dependent melanizing activity was observed only in a high mass (670kD) melanization complex (MC) significantly larger than PO. However, PO was essential for activity: (1) Egf1.0, a viral inhibitor of pro-PO processing, blocked the formation of the MC. (2) Immunoblots using a B. mori-specific PO antibody showed the formation of a high mass MC. (3) Trypsin digestion and mass spectrometry of the MC indicated the presence of PO. Overall, our finding that the substrate for melanin formation is Tyr and that it is metabolized solely by a PO-containing high mass MC undermines the long-held concept that PO activation alone is sufficient for melanization.

Keywords:*immunity, melanization*

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PS2M188

Physiology

P2

Effects of temperature and photoperiod on hypermetamorphosis in the been blister beetle, *Epicauta gorhami*

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Larvae of the bean blister beetle, Epicauta gorhami (Coleoptera: Meloidae), feed on grasshopper eggs in soil and undergo hypermetamorphosis. This beetle has larval diapause in the 5th instar as a pseudopupa, a form characteristic of hypermetamorphosis in meloid beetles. Effects of temperature and photoperiod on larval development of E. gorhami were examined in a population in Miyazaki, Japan. At 20 and 22.5ºC, all larvae became pseudopupae in the 5th instar, regardless of the photoperiod. In contrast, at 27.5 and 30ºC, almost all larvae pupated from the 4th instar, again regardless of the photoperiod. A long-day photoperiodic response occurred only at 25ºC: under 12L-12D, all larvae entered diapause, but the diapause incidence tended to decrease as the day length became longer. Pseudopupae were immobile and remained in diapause for a long period. Based on the short developmental period and diapause avoidance under summer conditions, it was suggested that this beetle partially produces two generations a year in southwestern Japan. Pseudopupal diapause was terminated by exposure to low or high temperature. The optimum low temperature for diapause termination was 10-15ºC. There was an individual variation in the low temperature requirement for diapause termination and post-diapause development may not occur until this requirement is satisfied. Although under natural conditions pseudopupae may encounter low temperatures that are effective for diapause termination at different ages, our results show that pseudopupae at various ages respond similarly to low temperature exposure. This response may ensure that resumption of development synchronizes in a population.

Keywords:*temperature, photoperiod, hypermetamorphosis, been blister beetle, Epicauta gorhami, pseudopupal diapause*

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Physiology P2

Larval development and diapause in the ramie longicorn beetle, *Paraglenea fortunei*

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The ramie longicorn beetle, Paraglenea fortunei (Coleoptera: Cerambycidae), feeds on weeds such as the ramie, Boeheria nivea, and trees such as the cotton rosemallow, Hibiscus mutabilis, in both the larval and adult stages. This beetle overwinters in the larval stage and emerges and reproduces in early summer. To examine seasonal adaptations of P. fortunei, effects of temperature (20, 25 and 30ºC) and photoperiod (12L-12D, 14L-10D and 16L-8D) on larval development were examined by rearing larvae after hatching using artificial diets in a local population in Miyazaki, Japan. Artificial diets used were Silkmate 2S, Insecta LFM, and Insecta F-II with dried leaf powder of the ramie (each commercial diet was from Nihon Nosan Co.). Body weight of the larvae varied with dietary conditions, but developmental profiles did not vary significantly among the conditions. The higher the temperature, the more times larvae underwent molts (mostly 3-6 times), but irrespective of the temperature and photoperiod, most larvae ceased feeding and did not pupate under constant conditions, except for the larvae that pupated precociously. When the larvae reared at 25ºC were exposed to lower temperatures (12.5 and 15ºC) for 30-60 days, a part of them pupated after return to 25ºC. These results indicate that this beetle has obligatory larval diapause that is terminated by low temperatures and thereby retains univoltine life cycles in the field.

Keywords:*larval diapause, ramie longicorn beetle, Paraglenea fortunei, artificial diet, low temperature*

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PS2M190

Physiology P2

How are odorant binding proteins involved in olfaction of *Tribolium castaneum?*

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The red flour beetle (Tribolium castaneum) is accessible to transgenic approaches as directed gene expression, powerful reverse genetics based on systemic RNA interference and a recently published full genome sequence. Moreover, its longevity offers a suitable system to address olfaction from the odor recognition and discrimination at the periphery to the analysis of the plasticity of the central olfactory pathway. According to genome annotation T. castaneum has got 47 odorant binding proteins (OBP) and 19 structurally related chemosensory proteins (CSP). Reverse genetic, electrophysiological and chemo-ecological approaches are combined to study the correlation of odorants to odorant binding proteins (OBP). The biological function of OBPs is still largely unknown, despite their necessity for olfaction. Especially the interaction of OBPs with odors is of key interest and is evaluated at the molecular and at the electrophysiological level.

Keywords:*Tribolium, castaneum, OBP, RNAi, EAG, olfaction*

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PS2M191

Physiology

P2

The effect of insect-virus interactions on the wheat metabolome under increasing atmospheric carbon dioxide

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Rising CO2 associated with climate change may affect the interactions between plants, pathogens and their vectors. Although plant response to elevated CO2 (eCO2) has been well studied, little is understood about the interactions between plants, insects and pathogens under simulated future climates. Subsequently, there is a lack of knowledge on how the plant and vector metabolome will respond to future atmospheric changes. Controlled environment growth chambers are being used to study the effect of eCO2 on this complex system. Experiments so far have examined the change in the metabolome of aerial wheat tissue due to infection by Barley yellow dwarf virus (BYDV) as transmitted by the aphid Rhopalosiphum padi under atmospheric CO2 and eCO2 (650ppm). Metabolomic analysis by LCMS profiling will be presented. Preliminary analysis reveals strong metabolic shift between control and virus infected plants with a more moderate effect due to eCO2.

Keywords:*climate change, vector, barley yellow dwarf virus, aphid*

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PS2M192

Physiology

P2

Juvenile hormone biosynthetic organs in the final larval instar of the red flour beetle *Tribolium castaneum*.

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Juvenile hormone acid O-methyltransferase (JHAMT) is an enzyme working in the final step of juvenile hormone (JH) biosynthesis. In Tribolium castaneum, RNA interference-mediated knockdown of JHAMT resulted in precocious larval?pupal metamorphosis due to precocious shutdown of JH biosynthesis. Generally, JHAMT is specifically expressed in the corpora allata, while existence of JHAMT mRNA in ovary and testis has been reported in the final larval instar in several insect species. It was found that JHAMT mRNA is expressed in the thorax or abdomen of last instar of T. castaneum (Minakuchi et al., 2008), but the tissue in which JHAMT mRNA exists has not yet been identified. In this study, temporal and spatial expression of JHAMT mRNA in the final larval instar was examined using quantitative RT-PCR. The amount of JHAMT mRNA in thorax-abdomen part was low until Day 3 of the final larval instar, and high in the prepupal stage. Further, the amount of JHAMT transcript in the thorax-abdomen of wandering larvae was relatively similar to the head containing the corpora allata. To identify the segments in which JHAMT is expressed, the thorax-abdomen was cut into anterior and posterior parts, then JHAMT mRNA was quantified. Although the JHAMT transcript level was relatively high in the posterior part of thorax-abdomen, it seemed that JHAMT was broadly expressed in thorax and abdomen. Further studies including in situ hybridization are in progress.

Keywords:*juvenile hormone, juvenile hormone acid O-methyltransferase, Tribolium castaneum*

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PS2M193

Physiology

P2

Insect hormones regulate the expression of diapause hormone receptor gene of the silkworm (*Bombyx mori*) In Vitro

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In the process of silkworm (Bombyx mori) diapause, the diapause hormone receptor (BmDHR) plays important roles. To study the regulation mechanism of BmDHR, two heterogeneous promoter fragments of Bmdhr were cloned into pGL3.0 basic vector to construct reporter plasmids, respectively. And the effects of foreign insect hormones including juvenile hormone analogue (JHA), 20-OH-ecdysone and diapause hormone (DH) on regulation of Bmdhr promoter activities were analyzed in BmN cells. The results showed that the activity of 1395 bp promoter fragment was significantly lower than that of 972 bp one, implying that there is a negative regulation element between -941 and -1364 nt of Bmdhr promoter region. JHA of 2, 4 and 6 μg/mL increased the promoter activity by 1.5-2.1 folds. The effects of 20-OH-ecdysone on Bmdhr promoter activity revealed a dosage dependent profile, low concentration 20-OH-ecdysone (1, 2 μg/mL) increased the promoter activity significantly, 20-OH-ecdysone of 4μg/mL strongly suppressed the promoter activity. When 20-OH-ecdysone concentration increased to 8 and 10 μg/mL, the promoter activity recovered and increased significantly again. Mixture of 1 μg/mL JHA and 20-OH-ecdysone increased the Bmdhr promoter activity by 1.6 folds to the peak. With the increase of mixture concentration the Bmdhr promoter activity decreased gradually, and high concentration (8 and 10 μg/mL) of mixture decreased the promoter activity. The effects of DH revealed a dosage dependent profile too, low concentration DH (10, 20 and 40 nmol?L-1) significantly enhanced the promoter activity, but high concentration DH (60 to 100 nmol?L-1) showed no significant effect.

Keywords:*Bombyx mori, Insect hormone, Diapause, Diapause hormone receptor,Gene promoter*

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PS2M194

Physiology P2

Nutrition and radiation influence protein expression on oriental fruit fly pupae

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Background: We reported on a study designed to identify molecular markers of deficiencies in culture media developed for the oriental fruit fly. In that study, we found significant changes in expression of 70 proteins in adults of larvae reared on media lacking wheat germ oil (WGO), compared to media supplemented with WGO and the WGO effect is highest at day 8 (females begin reproductive maturation). In a consecutive papers we addressed the idea that the whole animal proteome changes dynamically with different stages and ages (0-12 d). Methods: We reared separate groups of fruit fly larvae on media with and without WGO supplementation and the one with WGO was treated with different doses of radiations and analyzed protein expression in pupal age 0-11a days old using 2D electrophoresis. Results and discussion: Gel densitometry revealed significant increases (by > 2-fold) and decreases (by > 50%) in expression levels between 2-18 proteins from 1 to 11-d-old pupae respectively between the pupae of larvae reared on + and -WGO and between the pupae of larvae under radiation (IRR) and non-radiation (NIRR) treatment. Three major proteins, bnb (27-fold), nattlectin precursor (13.22 fold), and insect cuticle protein (12.90-fold) increased in expression in day 4, 6, 7 pupae were identified between +/-WGO. Conclusion: The key finding is that both nutrition deficiency and radiation treatment affect changes in protein expression started at day 4 pupae and these changes mainly occurred on developmental protein complex (bnb) located at molecular weight between 25-100 daltons and PI 4.5-5.

Keywords:*wheat germ oil, fruit fly diet, Bactrocera dorsalis, proteomics*

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PS2M195

Physiology

P2

Effect of plant secondary compounds on detoxification enzymes and oxidoreductases of cereal aphid

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Plant secondary metabolites have been documented to be deleterious to insects and other herbivores in diverse ways. In this paper, the effect of catechol (phenolics), gramine (alkaloid) and L-ornithine-HCl (non-protein amino acid) on the activities of detoxifying enzymes and oxidoreductases in Sitobion avenae was evaluated. Glutathione S-transferase (GST) and esterase (EST) activities in aphids were significantly or very significantly correlated with each compound concentration in artificial diets. Both two enzyme activities in aphids were significantly activated by gramine in comparison with catechol and L-ornithine-HCl, Peroxidase (POD) and polyphenol oxidase (PPO) activities in aphids were significantly correlated with Catechol and gramine concentrations in the diets, respectively, whereas catalase (CAT) activity in aphids was significantly suppressed by higher concentrations of catechol, gramine and L-ornithine-HCl. Those results indicated that EST and GST were important detoxification enzymes of aphids against plant secondary compounds, and suggested that POD and PPO could involved in aphid defense against phenolics and alkaloids, respectively, whereas three compounds could be inhibitors of CAT acitivity of aphids.

Keywords:*detoxifying enzyme, oxidoreductase, plant defense, plant secondary compound, Sitobion avenae*

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PS2M196

Physiology

P2

Induction of gene silencing in cultured insect cells by *piggyBac* transposon

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The piggyBac transposition system has been widely used for generating various transgenic insects and stably transformed insect cell lines for studying genetic mechanisms and developing new technologies for pest control as well as protein production. Due to a relatively random fashion of piggyBac integration into the genome, its insertion site and copy number among transformants considerably vary, resulting in significant differences in expression level of transgene. In fact, we have established several transgenic clonal lines with different levels of GFP marker gene expression including a few lines with no detectable expression by the piggyBac-mediated transformation of a silkworm cell line BmN4, which was derived from the ovary of Bombyx mori. Analyses of some GFP-negative lines have revealed that, from an endogenous PIWI-interacting RNA (piRNA) cluster (torimochi) of the B. mori genome, an antisense GFP sequence fused with a part of the cluster is commonly transcribed and serves as a source of de novo piRNAs capable of silencing GFP expression in trans (Kawaoka et al., 2012). The results indicate that the piggyBac can be used not only for gene expression but also for gene silencing by the piRNA pathway in BmN4 cells. We are further exploring the efficiency of endogenous gene silencing by the newly found piRNA-generating mechanism.

Keywords:*piRNA, piggyBac, Bombyx mori, BmN4, torimochi*

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PS2M197

Physiology

P2

Electroantennogram and field resoponses of the rice leaf folder, *Cnaphalocrocis medinalis*, to sex attractant candidates in Korea

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A series of studies was conducted to find out the optimum sex attractant for monitoring the rice leaf folder, Cnaphalocrocis medinalis, in Korea, based on the previously reported pheromone composition in this species. (Z)-13-Octadecenal (Z13-18:Al) was most active in electroantennogram (EAG) and field tests. (Z)-11-Hexadecenal (Z11-16:Al), (Z)-11-octadecenal (Z11-18:Al), (Z)-11-hexadecenyl acetate (Z11-16:Ac), (Z)-13-octadecenyl acetate (Z13-18:Ac) and (Z)-13-octadecenol (Z13-18:OH) individually elicited significant EAG responses, but the compounds were not attractive individually in fields. (Z)-11-Octadecenol (Z11-18:OH) alone was inactive in both EAG and field tests. The addition of Z11-18:OH and Z13-18:OH to the binary mixture of Z11-18:Al and Z13-18:Al, as the previously reported composition of Japanese blend increased significantly the trap catches of males in fields. In contrast, Philippine and Indian blends were not attractive to this species. Interestingly, when Z13-18:Ac alone was added to the binary mixture of Z11-18:Al and Z13-18:Al, the trap catch number was the same as that of Japanese blend. The present study indicates that the four-component blend (Z11-18:Al/Z13-18:Al/ Z11-18:OH/Z13-18:OH = 11/100/24/36) and the three-component blend (Z11-18:Al/Z13-18:Al/ Z13-18:Ac = 11/100/11) can be used as sex attractants for monitoring the Korean populations of C. medinalis.

Keywords:*Cnaphalocrocis medinalis, Korean population, sex attractant, EAG, field-test*

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PS2M198

Physiology

P2

Suppression of pheromone biosynthesis via RNA interference in *Plutella xylostella*

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Sex pheromone production is regulated by pheromone biosynthesis-activating neuropeptide (PBAN) in many lepidopteran species. A cDNA isolated from female adult heads of Plutella xylostella encodes 193 amino acids including PBAN, designated as Plx-PBAN. When female adults were injected with synthetic Plx-PBAN, pheromone production showed a maximal increase 1h post-injection. RT-PCR screening revealed that Plx-PBAN cDNA was expressed in all examined body parts, with the highest expression level in the head of female adults. The PBAN receptor (Plx-PBANr) gene was also cloned from the female pheromone gland. The expression of Plx-PBANr was found only in the pheromone gland of female adults among examined tissues and developmental stages. Heterologous expression in human uterus cervical cancer cells revealed that Plx-PBANr induced significant calcium elevation when challenged with Plx-PBAN. Female P. xylostella injected with double-stranded RNA specific to Plx-PBANr showed suppression of the receptor gene expression and exhibited significant reduction in pheromone biosynthesis, which resulted in loss of male attractiveness. In addition, to assess molecular events occurring downstream of PBAN signaling, partial sequences of Δ9 and Δ11 fatty acid desaturases of P. xylostella. were cloned. Phylogenetic analysis indicated that these two desaturase genes were highly clustered with other desaturases associated with sex pheromone biosynthesis in other insects. RT-PCR analysis showed that Δ9 desaturase was dominantly expressed in adult females, whereas Δ11 desaturase was expressed in all developmental stages. When PBANr expression was suppressed by PBANr-RNAi, the treated females also showed significant suppression of expression of both desaturases.

Keywords:*PBAN, PBAN receptor, RNA interference, desaturase, pheromone, Plutella xylostella*

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PS2M199

Physiology

P2

Identification of *Monochamus alternates* and *M.saltuarius*, the vectors of pimewood nematode, *Bursaphelenchus xylophilus* using internal transcribed spacer fragment in Korea.

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Monochamus alternatus(MA) and M. saltuarius(MS) were reported as the vectors of pinewood nematode, Bursaphelenchus xylophilus, in Korea. Aerial spray of pesticide in optimal occurrence period is the major way of prevention at pine wilt disease damaged area. To understand their regional distribution is the first step of prevention of the spread of pine wilt disease. However, it is not easy to identify them obviously because they are closely related not only biologically but also morphologically, in all developmental stages except adult. We designed PCR generic primer sets to amplify a segment of cytochrome oxidase subunit I (CO I) and internal transcribed spacer (ITS) and performed PCR with DNA extracted from larvae, pupae and adult of MA and MS. We cloned 850bp of CO I fragment and 1.3kbp of ITS fragment from 2 species. There were no distinctive differences between 2 species in sequence analysis of CO I. On the other hand, we developed species specific PCR primer sets to amplify ITS fragment of each Monochamus beetles and got the same result in larval, pupal and adult stage of each. These result shows that ITS is more proper method to identify 2 Monochamus beetles regardless of their developmental stages. In further study, we are planning to apply to other wood boring beetles in pine wilt disease damaged trees.

Keywords:*Monochamus alternates, Monochamus saltuarius, ITS, CO I*

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PS2M200

Physiology

P2

The molecular and behavioral effects of xenobiotic stress on immune and nervous systems of two honey bee species, *Apis mellifera* and *Apis cerana*

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Xenobiotic stresses induced by pesticides have been thought to disrupt the metabolic regulations in many organisms. The honey bee is an important social insect used for pollination in many crops, which has shown several symptoms of colony disruption caused by many environmental stress factors. In this study, we compared molecular and behavioral responses of two honey bee species, European honey bee, Apis mellifera and Asian honey bee, Apis cerana. These two honey bee species are likely to be exposed to pesticides-treated plants such as carbaryl in the field. Thus, here we investigated the effects of carbaryl on the regulation of gene induction in the immune and nervous systems as well as behavioral responses in A. mellifera and A. cerana. In combined with next generation sequencing analysis and learning behavioral paradigms in honey bees, we measured the expression levels of target genes involved in physiological stress and immunity. We also tested the proboscis extension response (PER) to determine the alternation of cognitive abilities in honey bees induced by carbaryl. This study will bring us better understandings of the impacts of xenobiotic stress factors on the immune and nervous system in two species, A. mellifera and A. cerana.

Keywords:*Apis mellifera, Apis cerana, xenobiotic stress, carbaryl, immune, nervous system, PER*

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PS2M201

Physiology

P2

Modulation of olfactory sensitivity and behavioral response to diet-induced stress in *Drosophila melanogaster*

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Obesity induced by consuming high-fat-diet (HFD) in humans and rodents is a main contributor to metabolic diseases such diabetes, hyperglycemia, hypertension, dyslipidemia, and cardiovascular diseases associated with insulin resistance. However, investigating effects and relationship between high-fat diet (HFD)-induced obesity and its olfactory sensitivity mediators have yet to be elucidated. To gain insights into how HFD-induced obesity affects olfactory sensitivity and neural behavioral responses, we here study Drosophila to test this hypothesis involving nutrient-sensing signal transduction pathways. Drosophila larvae reared on a high-fat diet showed hyperglycemic and insulin resistant. Also, they showed the increase of fat accumulations in the fat body compared with those reared on normal diets. Moreover, those larvae showed different patterns of food selection and locomotion behaviors, demonstrating the different level of gene expressions relevant to the homeostasis of lipid regulations. Remarkably, this modulation in feeding behaviors was accompanied with significant changes in olfactory sensitivity. Thus, this olfactory modulation induced by food stress in Drosophila might reflect functional variation and modification on feeding behaviors through nutrient-dependent metabolic sensing pathways.

Keywords:*obesity, high-fat-diet (HFD), sensitivity, feeding behavior, olfactory modulation*

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PS2M202

Physiology

P2

Inhibitiory effect of water extracts from *Acanthocoris sordidus* on oxidative damage by ROS via its antioxidant activities

Young Mi Park[1], Jae Hwan Lim[2], Jong Eun Lee[3], Eul Won Seo[4]

[1]Andong National University, Republic of Korea, [2]Andong National University, Republic of Korea, [3]Andong National University, Republic of Korea, [4]Andong National University, Republic of Korea

Present study aimed to evaluate the effect of water extracts from Acanthocoris sordidus on antioxidant and biological activities. Even water extracts from A. sordidus showed low antioxidant activities, but removed DPPH free radical and hydroxyl radical by 48.9%, 37.8%, respectively and 80.0% of ferrous iron chelating activities. In addition, we tested the reducing activities of water extracts against oxidative cell and DNA damage. The water extracts from A. sordidus showed a protective effect on the oxidative cell damage by inhibiting the lipid peroxidation by 92.4%. Also we determined an increased level of p21 protein expression by 68.1%. The inhibition of DNA fragmentation were 53.3% and phosphorylation level of H2AX was 30.1% compared to the radical experimental group. Taken together, we suggest that the water extracts from A. sordidus has a role as chemopreventor substances against oxidative damage.

Keywords:*Acanthocoris sordidus, ROS, antioxidant activities*

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PS2M203

Physiology P2

Effect of *Hydropsyche kozhantschikovi* extracts on oxidative cell and DNA damage

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In this study, we evaluated the protective effect of ethanol extracts from Hydropsyche kozhantschikovi on oxidative cell and DNA damage induced by free radical. Antioxidant activities of ethanol extracts from H. kozhantschikovi were low from scavenging activities of DPPH free radical and hydroxyl radical by 26.1% and 30.7% respectively and chelating ferrous iron by 26.2%. However, ethanol extracts from H. kozhantschikovi showed protective effects on the oxidative cell damage by inhibiting the lipid peroxidation by 99.2%, and showing an increased level of p21 protein expression by 92.2%. In addition, we tested the activities of extracts for reducing free radical-induced DNA damage. DNA fragmentation inhibition level in H. kozhantschikovi extracts was 70.3%, and inhibitory effect of phosphorylation of H2AX was 83.3% compared to the radical experimental group. In conclusion, although ethanol extract from H. kozhantschikovi showed a little antioxidant activities, it showed a strong protective effect on oxidative cell and DNA damage.

Keywords:*Hydropsyche kozhantschikovi, oxidative damage, radical*

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PS2M204

Physiology

P2

A microRNA-based screen for novel feeding-related target genes

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All organisms must match food intake with their metabolic needs. To do so, different species have developed feeding behaviors that vary widely in meal size, frequency, foraging strategy, and food preference. Well-regulated feeding is critical to growth, reproduction, and survival. To achieve appropriate balance, the brain must process internal information about nutrient stores and external information about available food sources and the immediate environment. We are interested in identifying novel genes and circuits involved in insect feeding behaviors. To do so, we have created a library of ~150 fly stocks that permit the tissue-specific overexpression of Drosophila microRNAs. MicroRNAs are single-stranded RNAs of approximately 22 nucleotides in length that play an important role in regulating gene expression by binding sequence-specifically to their target mRNAs and inducing their degradation. By crossing these UAS-miRNA lines to both fat body and pan-neuronal GAL4 drivers, we have identified miRNAs that enhance appetite in a modified Capillary Feeder (CAFE) assay. Oddly enough, the resulting prolonged overeating increases fly weight while reducing the size of fat body lipid droplets. Since all methods for bioinformatic prediction of miRNA targets are prone to generating false positives, we are carrying out a smaller secondary screen using UAS-RNAi lines for predicted targets to identify the specific target mRNA(s) being affected in the positive hits from the primary screen.

Keywords:*Drosophila, feeding, miRNA, fat body, behavior*

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PS2M205

Physiology

P2

Female sex pheromone components of the box tree pyralid, *Glyphodes perspectalis* in Korea: Field test and development of film type formulation

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Box tree pyralid, Glyphodes perspectalis, is the most serious pest of box tree in Korea, and was recently introduced into Europe. The pheromone components of this moth have been identified as (Z)-11-hexadecenal (Z11-16:Ald), (E)-11-hexadecenal (E11-16:Ald), and (Z)-11-hexadecenol (Z-11-16:OH). The ratios of these three compounds were 5:0.96:0.2, respectively. In field bioassay, (Z)-11-hexadecenal or (E)-11-hexadecenal alone was not attractive to males, but the mixture of Z11-16:Ald and E11-16:Ald was attractive to males. The most effective ratios of Z11-16:Ald and E11-16:Ald was 5:1 and 7:1. (Z)-11-hexadecenol was the strong antagonist in field bioassay. Small amount of (Z)-11-hexadecenol inhibited attractiveness of male moth. To make a film type formulation of Glyphodes perspectalis pheromone, mixture of photo-setting resin, the mixture of Z11-16Ald and E11-16:Ald (5:1) and BHT was added and then homogenized. The mixture of resin and pheromones was applied to a polypropylene film (23 × 24 cm OHP film, Uchida, Tokyo, Japan) and spread over the film by a bar coater. In field bioassay, film type pheromone was more attractive than septum type pheromone.

Keywords:*box tree pyralid, Glyphodes perspectalis, pheromone, film type formulation, field test*

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PS2M206

Physiology P2

A miRNA-based screen for novel genes involved in olfactory axon targeting

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One of the most interesting aspects of the Drosophila olfactory system is the incredible stereotypy with which it develops. The axons of sensory neurons expressing the same odorant receptors always manage to converge onto the same antennal lobe glomerulus. In order to identify novel genes that direct this precise and stereotyped connectivity of olfactory axons to their target glomeruli, we have generated a library of fly stocks that permit the tissue-specific overexpression of Drosophila miRNAs. Using the PNS driver pebbled-GAL4, we have overexpressed ~150 miRNAs in developing olfactory neurons as they enter the antennal lobe. We have identified 18 different miRNAs that disrupt olfactory axon guidance to the proper glomerulus. Since the available methods for miRNA target prediction are prone to generating false positives, we are currently carrying out a smaller secondary screen using UAS-RNAi lines for predicted targets to identify the specific target mRNA(s) that are being affected in the positive hits from our primary screen. It is our hope that this two-tiered screening method will expedite the identification of novel genes involved in olfactory axon guidance.

Keywords:*Drosophila, miRNA, olfaction, axon guidance*

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PS2M207

Physiology

P2

Why a membrane-anchored chitinase (CHT7) in *Tribolium?*

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Insect chitinases (CHTs), which belong to family 18 glycosylhydrolases, have been detected in molting fluid and gut tissues and are predicted to mediate the digestion of chitin present in the exoskeleton and peritrophic matrix in the gut. Based on amino acid sequence similarity and phylogenetic analysis, insect CHT family proteins have been classified into eight groups. CHT7s belong to Group III chitinase contain two catalytic domains and one chitin binding domain (CBD). This group of chitinases, unlike most insect CHTs, possesses a predicted transmembrane segment at the N-terminal region. The recombinant T. castaneum CHT7 that was expressed in Hi-5 insect cells was bound to the cell membrane. Apparently, the catalytic domains of this CHT face the extracellular space as revealed by its ability to hydrolyze an artificial chitin substrate added to the medium. DsRNA-based functional studies (RNAi) for several CHT genes in Tribolium castaneum indicated that CHTs belong to groups I (TcCHT5) and II (TcCHT10) are critical for molting and turnover of chitin in the old cuticle. In other hand, RNAi for TcCHT7 did not affect any types of molting such as larval-larval, larval-pupal and pupal-adult. The resulting pupae (and adults), however, failed to wing-expansion and abdominal contraction. Interestingly, dsTcCHT7 injection significantly reduced the cuticular chitin in the elytra and body wall. Transmission electron microscopy revealed that down-regulation of TcCHT7 resulted in disorganize the cuticular chitin laminar. These results suggest that TcCHT7 may have critical roles in the laminar assembly and synthesis and/or deposition of cuticular chitin.

Keywords:*Tribolium castaneum, Chitinase, Chitin, RNAi, cuticle, exoskeleton*

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PS2M208

Physiology P2

Temperature-dependent development of *Lymantria dispar* (Linne) (Lepidoptera: Lymantriidae)

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Lymantria dispar (Linne) (Lepidoptera: Lymantriidae) is known for minor pest and is thinly populated in korea, whereas It's major invasive species causing a lot of damage in the eastern part of the Canada and U.S.. We experimented that the established life history and temperature effect on the emergence of L. dispar adults using egg mass collected at last winter and stored in cold room. Rearing conditions were as fllows: 70% R.H., photoperiodic control 14L:10D and temperature set to 15, 20, 25 and 30°C. All the processes of life history of the species were as below. The female emerged at each temperature: 15°C; 116.02, 20°C; 75.24, 25°C; 56.18 and 30°C; 45.70 days. The male emerged at each temperature: 15°C; 117.90, 20°C; 70.03, 25°C; 50.08 and 30°C; 41.09 days. The adult emergence was early in higher temperature. The female emerged earlier than the male. The mortality was increase in higher temperature. Therefore, the results of this study should aid in quarantine inside Canada and U.S..

Keywords:*Lymantria dispar, gypsymoth, development temperature, rearing*

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PS2M209

Physiology

P2

Feeding behaviors of *Cacopsylla pyricola* (Homoptera: Psyllidae) and their changing feeding behaviors to insecticides

Min Woo Park[1], Shin Hyuk Jo[2], Sae Hee Kim[3], Hye Ri Kwon[4], Mi Ja Seo[5], Yong Man Yu[6], Young Nam Youn[7]

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The feeding behaviors of Cacopsylla pyricola and their changing feeding behaviors were recorded and analyzed with an electrical penetration graph (EPG) analysis against 5 insecticides. Feeding behaviors of C. pyricola monitored using the EPG technique; waveform PA which appears when it starts penetrates into the epidemic cell of pear leaf was occurred. And then waveform PB which its stylet secreted saliva was observed. Waveforms PC1 and PC2 involved the penetrating and sucking behavior in parenchyma cells. Otherwise, phloem salivation and ingestion behaviors produced waveforms PE1 and PE2. Ingestion in xylem tissue showed waveform PG. Among the feeding patterns of C. pyricola described above, phloem feeding pattern most frequently occurred, and xylem feeding pattern and parenchyma penetration pattern were appeared in the order. General feeding behavior patterns of C. pyricola were changed by insecticide treatments. The type and frequency of waveforms differently occurred depending on a sort of insecticides treated. In case of treatment of benfuracarb, total duration of non-probes (waveform Np) was appeared higher than any other insecticide. However, when flonicamid and deltamethrin were treatment, total duration of stylet penetration (waveform PA) and xylem ingestion (waveform PG) were appeared higher than other insecticide.

Keywords:*Cacopsylla pyricola, feeding behaviors, EPG pattern, insecticide*

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PS2M210

Physiology P2

Test for ability of degradation of intestinal bacteria of multicolored Asian ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae)

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The insect intestinal bacteria were isolated from the gut of the predacious multicolored Asian ladybird beetle, Harmonia axyridis, and then their ability of degradation to casein, chitin, cellulose and lignin was examined. Thirty-five isolates including genus Arthrobacter were isolated and identified from the gut of H. axyridis. Staphylococcus saprophyticus and S. sciuri were isolated and identified from all larval and adult stages. For the conformed the intestinal bacteria in Turnip aphid, Lipaphis erysimi that was provided to H. axyridis as prey, aphids grinded and checked. There are 5 strains including S. sciuri that was isolated same as H. axyridis. As a result of cultured intestinal bacteria on the media contained with skim-milk, chitin, cellulose and lignin, 12 strains were showed casein and 4 strains were chitin degradation. Otherwise, there was not found strains with cellulose and lignin degradation ability.

Keywords:*Harmonia axyridis, intestinal bacteria, casein, chitin, cellulose, lignin, degradation*

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PS2M211

Physiology

P2

Biochemical characterization of cold-adapted glycosyl hydrolases from the antarctic springtail acquired from *Bombyx mori*

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Endogenous Cellulases, laminarinase, and mannanase found in some plant, fungi, bacteria, and animals are β-1,4-Endoglucanases, β-1,3-Endoglucanases and β-1,4-D-mannanase that belong to the glycosyl hydrolase family (GHF). They have gained industrial interest due to their ability to modify and degrade cellulose-containing polysaccharides in bioethanol production and the food and feed industries. Recently, the freezing-intolerant Antarctic springtail is known to have various carbohydrases to digest the ingested food in the cold environment. Previously, we succeeded production of endogenous GHs of antartic springtail in E.coli, but it has not been accomplished mass production. Here, we report the successful mass production, purification, and characterization of C. antaricticus cellulase (CaCEL), laminarinase (CaLAM), and mannanase (CaMAN) using B. mori as host. The CaCEL produced in the silkworm were N-glycosylated and the innermost GlcNAc residue is linked to a α-1,3 Fucose residue. In specific lectin affinity assays, the affinity of N-linked glycan was different from those produced in cytosolic and heamolymph cells. The yield of the purified rGHs of springtail was evidently high, and the activated enzyme proved enough to give a positive signal during the in vitro activity assay. The results suggested a potential application for heterologous expression of cellulases in insect for biomass conversion. *This work was supported by Bio-industry Technology Development Program of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries.

Keywords:*Glycosyl Hydrolases*

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PS2M212

Physiology

P2

Molecular characterization and apoptotic mechanisms of psacotheasin, a novel knottin-type antimicrobial peptide, from *Psacothea hilaris*

Jae-Sam Hwang[1], Bomi Hwang[2], Juneyoung Lee[3], Dong Gun Lee[4], Eun-Young Yun[5], In-Woo Kim[6], Sung-Hee Nam[7], Seong-Ryul Kim[8], Mi-Young Ahn[9]

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We report the isolation and characterization of a novel knottin-type antimicrobial peptide from the yellow-spotted long-horned beetle Psacothea hilaris. A cDNA encoding a 56-mer knottin-type propeptide was identified and its predicted molecular mass and pI were 5.92 kDa and 8.28, respectively. A 34-mer mature peptide was also selected and named herein as psacotheasin. The antimicrobial activity of chemically synthesized psacotheasin against human bacterial pathogens was subsequently investigated. The results showed that psacotheasin exerted potent activities against both Gram-positive and Gram-negative bacterial strains. And we have further found that a series of characteristic cellular changes of apoptosis in C. albicans can be induced by the accumulation of intracellular reactive oxygen species, specifically hydroxyl radicals, the well-known important regulators of apoptosis.

Keywords:*Psacothea hilaris, knottin-type, antimicrobial peptide*

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PS2M213

Physiology

P2

The effects of paralization by *Bracon hebetor* Say (Hymenoptera: Braconidae) on hemolymph enzymes and proteins in larvae *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

Aydin Suzu Tuncbilek[1], Sevgi Bakir[2], Ebru Saatci[3], Guler Toprak[4], Ulku Canpolat[5], Tayfun Kaya[6]

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Activities of glutathione S-transferase (GST) and GST mu isozyme, catalase (CAT), superoxide dismutase (SOD) as well as hemolymph proteins of host larvae (Ephestia kuehniella, Lepidoptera: Pyralidae)) of Bracon hebetor (Hymenoptera: Braconidae) were determined. Tissues of 6 host larvae were pooled for each analysis and 3 replicates were performed for enzyme assays each treatment and measured by using spectrophotometer. Time courses after paralization influence on the antioxidant defense enzymes in host larvae as well as total hemolymph protein is evident. Specific activities of CAT, GST and GST mu isozyme activity reached its highest level in the eighth hour. These values were gradually decreased up to 8 and 24 h after paralysis to control sample level, respectively. Total hemolymph proteins levels showed fluctuation during course of time after paralization and reach highest value in 24 hours. Our work represents the first study of GST and CAT activities in the host larvae of B. hebetor that it is very useful for host-parasitoid relationships.

Keywords:*Bracon hebetor, Ephestia kuehniella, glutathione S-transferase, catalase, superoxide dismutase*

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PS2M214

Physiology

P2

Developing a molecular diagnosis key for five common cockroach species

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Cockroaches are of vital importance medically and hygienically as they can transmit human pathogenic agents. In this study, the genetic structure of five Iranian cockroaches including Periplaneta americana, Pycnoscelus surinamensis, Blattella germanica, Blatta lateralis and Blatta orientals has been examined using sequence analysis of mtDNA-COI gene to develop a molecular identification key for them. DNA was extracted and the barcode region of COI gene was amplified and sequenced using conserved primers. The sequences were analyzed to construct phylogenetic trees and to provide a physical map to introduce appropriate enzyme/s (RFLP markers) for molecular diagnosis of the five species. Inter-population variation (3%) was observed in P.americana but other species. Surinam cockroaches were the most diverged species (10%) among species. Cockroaches of Turkestani and Oriental at the DNA level and Turkestani and American at the amino acid level were the most similar species respectively. AluI enzyme by providing species-specific RFLP profiles could differentiate the species. This molecular marker, which is proposed to identify the five specimens, is new to science. Phylogenetic analysis revealed topography matched well with morphological classification. There was a very close relationship between oriental and turkestani cockroaches. P.americana populations maintain a high genetic diversity compared with other species. Seemingly, repeated exposure to mutational agents such as chemical poisons, local gene flow, and emigration from different parts of the world may the causes of such high genetic variations. Molecular key represented in this study can be applied for the molecular systematic of Cockroaches in scientific centers worldwide.

Keywords:*cockroach, mtDNA, COI, molecular systematics, RFLP molecular marker, Iran*

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PS2M215

Physiology P2

TYLCV acquisition influenced the attraction of whiteflies Bemisia tabaci to the green light emitting diodes (LEDs)

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Light attraction of whiteflies and thrips was compared using LED light bulbs that exhibit different wavelengths of visible and UV ranges. Whiteflies, both Bemisia tabaci and Trialeurodes vaporariorum, are mostly attracted to green LED (526 nm) while thrips, Frankliniella occidentalis, attracted to UV LED (371 nm). The attraction of green LED was higher in B. tabaci than T. vaporariorum while its rate was not different between B biotype and Q biotype of B. tabaci. We demonstrated whether TYLCV acquisition influences on the light attraction of B. tabaci. TYLCV infected B. tabaci was 4 times higher in the attraction to the green light LED than TYLCV free ones. Those attraction rates were significantly different in a 0.5 m distance to the light source but were similar at more than 1 m distance. Our results show that B. tabaci favors green light and its attraction is highly enhanced by the acquisition of a plant virus, TYLCV.

Keywords:*Bemisia tabaci, Green LED, Incandescent light, TYLCV, whitefly*

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PS2M216

Physiology

P2

Up-regulation of temperature susceptibility in *Bemisia tabaci* upon acquisition of *Tomato Yellow Leaf Curl Virus* (TYLCV)

Joseph Carlo A. Pusag[1], S. M. Hemayet Jahan[2], Kwan-Suk Lee[3], Sukchan Lee[4], Kyeong-Yeoll Lee[5]

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Acquisition of plant viruses has various effects on physiological mechanisms in vector insects. Bemisia tabaci is the only known vector of Tomato yellow leaf curl virus (TYLCV), which is a serious virus affecting tomato cultivars. In this study, the lifespan of Q1 biotype was compared between non-viruliferous (NV) and TYLCV-viruliferous (V) whiteflies. Total lifespan from egg to adult death was 10.64 days shorter in V than NV whiteflies. We investigated the temperature susceptibility of B. tabaci by comparing mortalities as well as heat shock protein (hsp) mRNA levels between NV and V whiteflies. For this, NV and V whiteflies were exposed for either 1 or 3 h at 4, 25, and 35°C. The mortality of V whiteflies was higher than NV ones following exposure at either 4°C or 35°C, but there was no significant difference at 25°C. Analysis of the expression level of heat shock protein (hsp) genes using quantitative real-time PCR showed that both cold and heat shock treatments stimulated higher expression of hsps (hsp40, hsp70, and hsp90) at various rates in V whiteflies than NV ones, but there was no difference at 25°C. All together, our results show that TYLCV acquisition accelerated the developmental rate and increased susceptibility to thermal stress in B. tabaci. Therefore, this modification may result in reduced vector longevity due to increased metabolic energy utilization. Our results provide insights into the complex interaction between vector fitness and thermal stress during the acquisition and transmission of plant viruses.

Keywords:*Bemisia tabaci, Heat shock proteins, Plant virus, TYLCV, Whitefly*

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PS2M217

Physiology

P2

The induction and termination of larval diapause in *Lycaena dispar*

Seong-hyun Kim[1], Namjung Kim[2], Seong-Jin Hong[3], Young-Bo Lee[4], Hae-Chul Park[5], Yeon-Ho Je[6]

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This study investigated the effects of photoperiod and temperature on larval diapause in the Large Copper butterfly, Lycaena dispar. This information was collected for use in establishing an indoor-rearing system for the Large Copper. Lycaena dispar requires 3~4 generations to complete its life cycle due to larval diapause. The induction of larval diapause was studied in this species for different combinations of temperature (15, 20, 25°C) and photoperiod (LD 14:10, 12:12, 10:14, 8:16). Most larvae entered diapause under short-day conditions but developed without diapause under long-day conditions (LD 14:10). However, 72% of the larvae entered diapause under long-day conditions (LD 14:10) at 15°C. The average developmental periods (>25 days) of larvae at 15°C were longer than those (<13 days) at 25°C. The duration of diapause induced by LD 10:14 at 15°C was significantly longer than the duration of diapause induced by other conditions. An examination of the sensitivity of immature larvae to low temperatures revealed that 1st instars showed susceptibility if diapause was induced by short-day conditions (LD 8:16, 20°C). To compare the effects of temperature and total periods of chilling on the termination of diapause, larvae were subjected to different chilling temperatures (3, 5, and 8°C) from 60 days of age. The rate of termination of diapause at 3 and 8°C was significantly higher than that at 20°C. However, pupation differed significantly among insects treated at 8°C.

Keywords:*Larval diapause, Diapause termination, Lycaena dispar, O2 consumption*

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PS2M218

Physiology

P2

Cloning and sequence analysis of a GH10 Endo-β-1,4-xylanase gene of *Streptomyces mexicanus* DY31, a bacterium in the gut of a mole cricket, *Gryllotalpa orientalis*

Do Young Kim[1], Hyo Jeong Kim[2], Han-Young Cho[3], Yi-Joon Kim[4], Dong-Ha Shin[5], Kwang-Hee Son[6], Ho-Yong Park[7]

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A hemicellulolytic bacterium capable of degrading β-1,4-xylans and β-1,4-mannans, Streptomyces mexicanus DY31, was isolated from the gut of a mole cricket, Gryllotalpa orientalis. In this study, we cloned and molecularly analyzed a novel, extracellular endo-β-1,4-xylanase (XylZ) gene of the bacterium by repeated genomic walking and nested PCR protocols. The cloned gene (1350-bp) was predicted to encode a polypeptide consisting of 449 amino acids with a deduced molecular mass of 46,798.8 Da and a calculated isoelectric point of 6.24. When analyzed by the SignalP 3.0 server, the enzyme was also predicted to have a unique N-terminal signal sequence (from Met1 to Ala30) of 30 amino acids. The results of a protein blast survey showed that XylZ was a modular endo-β-1,4-xylanase comprised of a catalytic GH10 domain (from Tyr44 to Leu332) in the N-terminus region and a carbohydate-binding module II (from Cys351 to Gly444) in the C-terminus region, respectively. This domain architecture of XylZ was most similar to that of Streptomyces lividans TK24 endo-β-1,4-xylanase (GenBank accession no.: EFD71285). However, the maximum sequence identity (85%) was oberved when the catalytic GH10 domain of XylZ was compared to that of Streptomyces sp. SirexAA-E endo-β-1,4-xylanase (GenBank accession no.: AEN08210), which has not yet been biochemically characterized.

Keywords:*endo-β-1,4-xylanase, GH10, Gryllotalpa orientalis, mole cricket, Streptomyces mexicanus*

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PS2M219

Physiology P2

Indoor-rearing system of the emma field cricket, *Teleogryllus emma*

NamJung Kim[1], Seong-Hyun Kim[2], Seong-Jing Hong[3], Young-Bo Lee[4], Seok-Jo Whang[5], Hae-Chul Park[6]

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To study the ecological characteristics of the emma cricket, Teleogryllus emma, development, reproduction, oviposition, and diapause of T. emma were investigated. The developmental periods of T. emma nymphs had a range of 124.8 days to 44.4 days at 21℃ and 35℃, respectively. At 15℃ and 18℃, however, all tested individuals died before emergence. The highest average fecundity per female was 737.3 at 25℃. In order to study the added effect of photoperiod, the nymphs of T. emma were reared under various photoperiods. The mean nymphal stages were 65 days in a 0-hour photoperiod, 49-50 days in an 8-, 10-, 12-, or 13-hour photoperiod, 59 days in a 14-hour photoperiod and 64 days in a 16-hour photoperiod. The nymphs matured faster in the short compared to the long photoperiods. Egg-forming and preservation methods were essential for a year-round rearing system of T. emma eggs, as T. emma undergo one generation per year. An oviposition preference experiment using different mats, soil (natural oviposition mat) and oasis® (artificial oviposition mat) showed that T. emma preferred to oviposit on the oasis® mat. Changes in volume, oxygen consumption, and water content in T. emma eggs were followed from oviposition. An increase in volume, oxygen consumption and uptake of water occurred from 7 to 8 days following oviposition. Oxygen consumption of eggs increased slowly for 7 days following oviposition, but decreased until 15 days following oviposition. These results showed that a physiological change at diapause initiation was reflected upon volume, water content, and oxygen consumption of

Keywords:*Cricket, development, reproduction, Diapause, Teleogryllus emma*

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Physiology P2

Purification and characterization of a β-1,4-mannanase from an intestinal bacterium of *Moechotypa diphysis, Paenibacillus* sp. Strain HJ-8

Hyo Jeong Kim[1], Do Young Kim[2], Han-Young Cho[3], Sung Young Jung[4], Yi-Joon Kim[5], Dong-Ha Shin[6], Kwang-Hee Son[7], Ho-Yong Park[8]

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A fibrolytic Paenibacillus sp. strain HJ-8 was isolated from the gut of a wood-attacking longicorn beetle, Moechotypa diphysis. This microorganism was able to efficiently decompose hemicellulosic materials such as β-1,4-xylans and β-1,4-mannans, whereas it could not degrade cellulose. In this study, we purified and characterized an extracellular β-1,4-mannanase (ManA) produced by the bacterium that was grown on a galactomannan (locust bean gum). ManA was purified by two consecutive column chromatography using Phenyl-Sepharose and Superdex 200 resins, respectively. The N-terminal amino acid sequence and molecular mass of ManA was analyzed to be APSFAVGADFSYVPG and 31.0 kDa. The enzyme displayed its maximum catalytic activity toward locust bean gum at 55oC and pH 7.0. In addition, the half-life of ManA at 55oC was evaluated to be approximately 25 min. The enzyme was capable of readily decomposing different mannan compounds such as locust bean gum, guar gum, ivory nut mannan, and mannooligosaccharides, while it had no effect on cellulose, soluble starch, β-1,4-xylans, p-nitrophenol (PNP)-glucopyranoside, PNP-xylopyranoside, or PNP-cellobioside. The results of HPLC analysis revealed that ManA could degrade locust bean gum to a series of oligosaccharides [M1 (0.5%), M2 (1.5%), M3 (2.5%), M4 (9.6%), M5 (54.1%), and M6 (31.8%)]. ManA was tightly bound to insoluble ivory nut mannan and Avicel, whereas it did not exhibit any binding capacity towards other hydrophobic materials such as insoluble oat spelt xylan, lignin, chitin, chitosan, and curdlan.

Keywords:*β-1,4-mannanase, Moechotypa diphysis, Paenibacillus sp. HJ-8, gut*

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Physiology P2

Development of a mycoinsecticide formulation with *Paecilomyces lilacinus* HY-4 for the control of Tetranychus urticae

Zhen Ji[1], Han-Young Cho[2], Do Young Kim[3], Yi-Joon Kim[4], Dong-Ha Shin[5], Kwang-Hee Son[6], Ho-Yong Park[7]

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Three types of propagules including aerial conidia, blastospores, and mycelia were employed to evaluate the shelf-life and thermostability of a biological control strain Paecilomyces lilacinus HY-4, which was used for manufacture of the mycoinsecticide to reduce the amount of conventional chemical pesticides in crop production systems. Compared to the high germination rate (>95%) of aerial conidia after 120d at ambient temperature (20±3oC), blastospores gave a survival rate of 12.7% only, and no germination was found in mycelia after 90d of storage. Therefore, the aerial conidia harvested through solid state fermentation by using inexpensive substrate rice bran were served for the wettable powder formulation of fungal pesticide- PaeciPora®. Bioassay trials were conducted on greenhouse-grown cucumbers, our results demonstrated the formulation exhibited a control efficacy of 63.7% against the adult females of Tetranychus urticae at 1×107 conidia/mL after 7d. This study indicates the novel mycopesticide PaeciPora® may be considered as promising for a new approach to control T. urticae in organic integrated pest management.

Keywords:*Paecilomyces lilacinus, PaeciPora, propagule, bioassay, Tetranychus urticae, IPM*

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PS2M222

Physiology

P2

Increase of 30K protein in identified motoneurons by hemolymph results in inhibition of programmed cell death in silkworm, *Bombyx mori* (Lepidoptera, Bombycidae)

Hwa Young Song[1], Mi Young Kim[2], Bong Hee Lee[3]

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This study demonstrates that a 30K protein was gradually synthesized in primary-cultured motoneurons from the accessory planta retractor (APR) of the 6th abdominal ganglion (APR6) in silkworm ventral ganglia through stimulation of hemolymph. An increase in 30K protein synthesis resulted in an inhibition of programmed cell death (PCD) of APR6 motoneurons. The 30K protein was gradually synthesized from the 30Kc6 gene of identified APR6s in day-6 4th instars to day-9 5th instar larvae, but synthesis of the 30K protein ceased in isolated APR6s of day-1 pupa, which normally begin to undergo PCD. When pupal APR6s were treated with larval hemolymph, however, the 30K protein was synthesized suggesting the existence of an anti-PCD factor in the larval hemolymph. An increase of 30K protein within the APR6s was confirmed by antiserum made against the recombinant 30K protein that originated from the APR 30Kc6 gene. Larval APR6, in which PCD was induced with 20-hydroxyecdysone (20E) added to the primary culture, exhibited some PCD characteristics of shrinkage of cell bodies, axonal fragmentation and loss of mitochondrial function. These results provide new insights on the survival or PCD of insect motoneurons through stimulation of hemolymph

Keywords:*programmed cell death, 20-gydroxyecdysone, 30K proteim, accessory planta retractor motoneuron*

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PS2M223

Physiology P2

Transcriptomic study of *Tenebrio molitor* larva that was treated with acholeplasma lysate injection

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To investigate genes related to innate immune system in T. molitor larva that was treated with acholeplasma lysate, we constructed cDNA library and analyzed expressed sequence taq (EST) sequences. All the sequences determined by single-pass sequencing were trimmed from the vector region and the low-quality sequences. We collected 1966 unique sequences composed of 1340 singletons and 254 contigs with an average 727 bp in untreated group and 1962 unique sequences composed of 1348 singletons and 240 contigs with an average 661 bp in treated group. These T. molitor EST sequences were searched against NCBI nr database by local BLAST (blastx, E<e-5). The 1726 putative sequences and 1732 putative sequences had significant hits within the database. To predict the potential functions of the genes, KOG (clusters of orthologous groups for eukaryotic complete genomes) analysis was conducted (blastx, E<e-10). As a result, 1154 genes and 1218 genes were significantly matched. Of these, most of genes were belongs to O category (Posttranslational modification, protein turnover, chaperones). Here, we identified up-regulated and/or down-regulated genes by acholeplasma lysate injection. Our results would be helpful to further study of innate immune mechanism in conjunction with mycoplasma.

Keywords:*Mycoplasma, ESTs, innate immunity, Tenebrio molitor larva*

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Physiology

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Genome sequencing and analysis of *Pieris rapael*> granulovirus

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The Korean isolate of Pieris rapae granulovirus (PrGV) genome was sequenced and analyzed. The double-stranded circular genome contains 108,658 bp and potentially encodes 120 predicted open reading frames (ORFs). BLAST searches followed by a comparison of ORF arrangement with those of completely sequenced granulovirus genomes showed the presence of 78 homologs to other genes in the database. In addition, three uncharacterized ORFs were observed and relate to ORF 9, 32 and 117 in both the Korean and Chinese isolates of PrGV in comparison with other sequenced GV genomes. The analysis predicted 120 ORFs with a size of above 50 amino acid residues showing minimal overlap. The 120 putative ORFs covered 92% of the sequenced genome. The genome had an A+T content of 66%, similar to the sequenced Chinese isolate and was close to CrleGV and ChocGV, with the highest A+T content of 67.6% and 67.3% respectively. The genomes of both the Korean and Chinese isolate of PrGV exhibit extensive sequence similarity and co-linearity and the identity figures reflect almost 99% homology. The mean overall amino acid identity of PrGV ORFs with other GV genomes was highest with ChocGV ORFs at 58%. The 120 predicted ORFs were grouped according to function into 10 genes involved in transcription, 11 genes involved in replication, 21 structural protein genes, 14 auxiliary genes and 61 unknown genes. Chitinase (ORF 10) and cathepsin (ORF11) genes were found in the genome, explaining the typical degradation patterns of the infected Pieris rapae larvae. The granulin gene (ORF 1)

Keywords:*rapae granulovirus, viral genome structure, viral gene expression pattern*

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Physiology

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CSP and takeout genes modulate the aggregation behavior in locust

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The migratory locust, Locusta migratoria, is a worldwide agricultural pest whose outbreaks can result in plagues during which they mass migrate in marching bands and flying swarms. A key step in the formation of large aggregation is the initial shift from the strong mutual aversion in solitarious locusts to the attraction to each other in gregarious locusts. This behavioral plasticity defines the most striking trait in locust phase change. However, the genetic basis for behavioral plasticity in locusts is largely unknown. To unravel the molecular mechanisms underlying the behavioral phase change, we developed a large-scale oligonucleotide microarray to quantify the expression of 9154 genes during the time courses of isolating gregarious locusts and crowding solitarious locusts. We found that several olfaction-related genes, CSPs and takeout, are strongly regulated in a total of 1,444 differentially expressed genes during both processes and display opposite expression trends in response to population density change. Tissue expression experiments revealed that CSPs and takeout genes exhibited antenna-rich expressions. RNA interference combined with olfactory behavioral experiments confirmed that the CSPs and takeout genes are involved in the rapid switch from repulsion to attraction between individuals during gregarization and the reverse transition during solitarization. These findings suggest that the response to locust-emitted olfactory cues regulated by CSP and takeout genes is involved in the behavioral phase change in the migratory locust and provide a previously undescribed molecular mechanism linked to the formation of locust aggregations.

Keywords:*Locusta migratoria, microarray, olfactory perception, aggregation behavior*

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Physiology

P2

Roles of a chitinase gene (*LmCht10*) in *Locusta migratoria* development

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Insect chitinases (EC 3.2.1.14, endochitinases) are enzymes catalyzing random hydrolysis of β-1-4 glycosidic bonds in chitin and chitodextrins. Multiple chitinase genes have been identified in various insect species, but their roles in insect development have not been well elucidated. We identified a chitinase gene (LmCht10) from Locusta migratoria, which was highly expressed in the integument. By using RNA interference in different nymph stages, we observed that nymphs injected with dsRNA for LmCht10 developed slowly, failed to detach from the old cuticle during molting process and showed high mortality (>95%). When the expression of LmCht10 was suppressed by RNAi, we observed significantly retarded new cuticle development and cuticle thickness as compared to the dsRNA GFP-injected controls. We further found that the developmental expression patterns of LmCht10 were correlated well with the change in 20-hydroxyecdysone (20E) titers from day 1 of the fourth-instar nymph stage to the 7-day-old adult stage. LmCht10 was quickly up-regulated after 20E was injected into the 2-day-old fifth-instar nymphs. Injection of cycloheximide (CHI) along with 20HE did not abolish the up-regulation of expression of LmCht10 indicating that new protein synthesis was not required for the elevated expression of this gene by 20HE. In contrast, LmCht10 was dramatically down-regulated in day 5, 6 and 7 of fifth-instar nymphs after RNAi of ecdysone receptor in day-2 fifth-instar nymphs. These results suggest that LmCht10 is a target gene for 20-HE and that its expression is directly induced by this hormone during nymph development.

Keywords:*chitinase, RNAi, function, hormone regulation, Locusta migratoria*

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Physiology

P2

Endocrine regulation of the peripheral clock gene expression in an insect model

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The linden bug Pyrrhocoris apterus exhibits a robust diapause response to photoperiod. Daily changes in the circadian clock gene expression were very week, but photoperiod strongly affected the basal levels of gene transcripts via the neuroendocrine system. In the gut, cryptochrome (cry) mRNA levels were much higher (about 10x) in diapause-promoting short days (SD) than in reproduction-promoting long days (LD), while Par Domain Protein 1 (Pdp1) mRNA levels were higher in LD than in SD. The effect of photoperiod on gene expression was mediated by the insulin producing neurosecretory cells of the pars intercerebralis (PI) and the juvenile hormone (JH) producing corpus allatum (CA). In LD-females, CA-ablation resulted in SD-like levels of gene transcripts, while PI-ablation had very little effect. In SD-females, the situation was opposite; PI-ablation resulted in LD-like levels of gene transcripts, while CA-ablation had no effect. Thus the CA is responsible for LD-like characteristics of gene expression in reproducing females and the PI (probably via inhibitory effect on the CA) is responsible for SD-like characteristics of gene expression in diapausing females. Levels of period (per) mRNA were not significantly affected by hormonal conditions. In contrast to the gut, cry mRNA levels in the fat body were not influenced by photoperiod, but levels of per and Pdp1 transcripts were inversely affected by contrasting photoperiods and the CA. Our results indicate that the peripheral clock gene expression reflects rather the physiological state of females (with respect to diapause or reproduction) than the external light-dark cycle. Acknowledgements: Grant GACR P502/10/1612

Keywords:*circadian clock genes, diapause*

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Physiology P2

Common and distinct juvenile hormone signaling pathways regulate metamorphosis and reproduction in hemimetabolous insects

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Juvenile hormone (JH), produced by the corpus allatum gland (CA), prevents metamorphosis in insect larvae and stimulates vitellogenesis in adult females. Whether both processes rely on the same JH signaling pathway is presently unknown. We examine the function of three JH-signaling genes: Methoprene-tolerant (Met), Taiman, and Kruppel-homolog 1 (Kr-h1) using systemic RNAi in larvae and adults of the hemimetabolous firebug, Pyrrhocoris apterus. In this species, Met conveys the JH signal to prevent premature metamorphosis by maintaining high expression of Kr-h1 (1). In contrast to Met and Kr-h1, injection of Taiman dsRNA did not produce premature adult development. In adult females of P. apterus, knockout of either Met or Taiman suppressed both vitellogenin synthesis and ovarian development. A similar effect was produced by ablation of the CA or by a natural absence of JH during reproductive diapause. Stimulation of vitellogenesis by treatment of diapause females with a JH mimic methoprene required both Met and Taiman. In contrast to Met and Taiman, injection of Kr-h1 dsRNA did not prevent stimulation of vitellogenesis by JH. Our results indicate that JH signaling pathways in larvae and adults of P. apterus include both common and distinct components. While the JH receptor Met operates in both larvae and adults, its partner Taimain is only required for vitellogenesis but not for the maintenance of the larval program. Conversely, Kr-h1 blocks adult development but is dispensable for vitellogenesis. 1 Konopova B., Smykal V., Jindra M. (2011) Plos ONE 6 (12): e28728 Acknowledgements: Grant GACR P502/10/1612

Keywords:*juvenile hormone signaling genes, metamorphosis, reproduction*

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Physiology

P2

A new coelomic gregarine parasite from the ghost moth, *Thitarodes pui* (Lepidoptera: Hepialidae)

Zixuan Sun[1], Wenjing Wu[2], Guren Zhang[3]

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Gregarines, a variety of apicomplexan parasites, inhabit the intestine, coelom or reproductive vesicle of invertebrates including insects. Recently, we discovered a coelomic gregarine parasite from Thitarodes pui distributed at 4,156~4,600m in the Tibet Plateau. The ghost moth is known as a host species of entomopathogenic fungus Ophiocordyceps sinensis with medical and economical importance. Visible gregarine gametocysts were found in the coelom of T. pui adult hosts, containing tens of thousands of biconical oocysts. In this work, lots of trophozoites were found in the intestinal lumen of T.pui larvae fed with 10^3 oocysts/larva. Young trophozoites were characterized by a protomerite-deutomerite septum and a flowerlike epimerite. The septum was lost in mature trophozoites (obovoid, 40~100 μm). Morphological characters of oocysts and trophozoites suggest that the gregarine species is closely related to the genus Paraschneideria, which is consistent with previous phylogenetic analysis inferred from SSU rDNA sequences.

Keywords:*coelomic gregarine, Thitarodes pui, parasite, Paraschneideria*

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PS2M230

Physiology

P2

Proteomic analysis of Sesamia nonagrioides brain in diapausing and non-diapausing developed larvae.

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The Mediterranean corn borer, Sesamianonagrioides, is a noctuid that feeds on maize, sugar cane and sorghum in North Africa and Southern Europe. Larvae reared under long day conditions pupate after 5 or 6 larval instars, whereas under short day conditions enters diapause and undergo up to 12 molts before dying or pupating. The role of the brain on the diapause induction is not still clear enough as debrained developed larvae displays several extra larval molts and maintain a noticeable titer of juvenile hormone in their hemolymph. To better understand the mechanism of larval development and diapause we identified proteins with different expression between diapausing and non diapausing larvae. Two- dimensional gel electrophoresis was employed to analyze the protein patterns of the brain of the sixth instar larvae, 2 or 7 days after molt. The spots showing differential expression were analyzed by mass spectrometry (MALDI-TOF MS or MALDI-TOF/TOF MS/MS). This study shows a large number of functionally proteins families in the brain of S. nonagrioides. The proteins expression at the brain in a specific time suggests that different developmental stages need specific proteins to be involved in.

Keywords:*Proteomic analysis, brain, 2D electrophoresis, mass spectrometry, Sesamianonagrioides.*

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Physiology P2

Molecular cloning and analysis of a novel beta-1,3-glucan recognition protein from the ghost moth, *Thitarodes pui* (Lepidoptera, Hepialidae)

Wenjing Wu[1], Zixuan Sun[2], Min Zhang[3], Chao Song[4], Guren Zhang[5]

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Detection of pathogen surface molecules is the crucial first step of host defense reactions. Beta-1,3-glucan recognition proteins (βGRPs) are soluble pattern recognition proteins that bind to β-1,3-glucans from fungi cell walls and initiate immune responses in insects. A novel βGRP gene was cloned from the ghost moth Thitarodes pui, narrowly distributed at an altitude of 4,100 to 4,650 m in the Mt. Sejila of the Tibetan Plateau. The βGRP was classified into βGRP clade 4 in phylogenetic analysis and designated TpβGRPc. The full-length TpβGRPc cDNA (1,915 bp) has a 1,464 bp open reading frame encoding a protein of 488 amino acid residues including a 21 amino acid residues signal peptide, a 127 bp 5' untranslated region (UTR) and a 324 bp untranslated region in the 3' UTR with a 26 bp polyA tail. The calculated molecular mass of the mature protein is 52,596 Da with an estimated pI of 6.06. Tertiary structure of TpβGRPc contains two distinct regions. The N-terminal domain responsible for β-1,3-glucan binding affinity includes ten putative β-strands. The C-terminal domain shows a classical sandwich-like β-jelly roll fold that is similar to β-1,3-glucanases. In phylogenetic tree, TpβGRPs (TpβGRPa, TpβGRPb and TpβGRPc) formed a distinct and separate clade 4 in Lepidopteran βGRP family, which might ascribe to the local distribution of the homoneurous primitive ghost moth.

Keywords:*Thitarodes pui, β-1,3-glucan recognition protein, tertiary structure, phylogenetic tree*

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Physiology

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The cabbage moth modulates the adult eclosion time in response to the amplitude of temperature cycle

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Adult eclosion rhythm of the cabbage moth, Mamestra brassicae, pupating in the soil, was recorded in darkness under two thermoperiods, 24°C (12h):16°C (12h) and 20.5°C (12h):19.5°C (12h), with different amplitude and the same average temperature. Clear daily rhythms of eclosion were observed in both conditions. However, moths eclosed about 2h before the tempeture drop in 20.5°C:19.5°C, but 4h later in 24°C:16°C. This response to the amplitude of temperature cycle, together with similar previous observations of adult eclosion rhythms in dipterous insects, might suggest a widespread means of adaptation among insects pupating in the soil to compensate for the depth-dependent phase delay of the temperature cycle that is the only time cue available in the soil.

Keywords:*Circadian rhythm, Thermoperiod, Temperature difference, Mamestra brassicae, Lepidoptera*

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Physiology

P2

Effects of knockdown of the adipokinetic hormone receptor on nutritional levels and feeding frequency in the two-spotted cricket *Gryllus bimaculatus*

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Adipokinetic hormone (AKH) is a crucial peptide hormone in insects that modulates the nutritional homeostasis by mobilizing lipid and carbohydrate. In this presentation, to address how imbalanced nutrientional levels in hemolymph would influence feeding behavior, we manipulated the expression level of AKH receptor in the two-spotted cricket, Gryllus bimaculatus. First, we cloned a cDNA encoding the receptor for AKH in this species (GrybiAKHR). Ca-imaging using Hek293 cells expressing GrybiAKHR confirmed that GrybiAKHR functions as an AKHR. Next, we performed phynotypic analyses using AKHR-downregulated crickets by RNA interference (RNAi) targeting GrybiAKHR. Knockdown of GrybiAKHR decreased hemolymph levels of 1, 2-diacylglycerol and trehalose, while it increased the level of triacylglycerol in the fat body, probably due to the consequent reduction of AKH signaling. In addition, observation in GrybiAKHR knockdowned crickets revealed three characteristic behavioral changes; (1) enhanced starvation resistance, (2) decreased locomotor activity, and (3) increased feeding frequency without changing meal duration. These data led us to a conclusion that the increased feeding frequency in AKHR-knockdowned crickets is affected by decreasing the levels of hemolymph lipid and carbohydrate below a certain threshold for maintenance the continuous nutrient demands. We also concluded that the nutrient levels in hemolymph which are partly controlled by AKH signaling affect feeding behavior, particularly feeding frequency. Trade-off between status of the economized expenditure and the status of the mobilizing energy source from the fat body might take place via AKH-signaling.

Keywords:*adipokinetic hormone, feeding behavior, Gryllus bimaculatus, energy metabolism*

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PS2M234

Physiology

P2

Identification and characterization of α-amylase in the gut of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae)

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The Red Palm Weevil, Rhynchophorus ferrugineus (Oliver) (Coleoptera: Curculionidae), is a serious pest of a wide range of plant species including coconut, sago, date and oil palms. It is native to Middle East countries where it is called Asian Palm Weevil, and it has been widely distributed in Southern Asia. Thus, the aim of the current study was to identify and characterize α-amylase activity to gain a better understanding of digestive physiology of the insect. Thus, the α-amylase in the gut of red palm weevil was isolated and characterized using starch as a substrate. For experiments, last instar larvae were collected from the date fields from Saravan and their gut was removed and homogenized in distilled water. The samples then were centrifuged and their supernatant was separated and kept as an enzyme source. The α-amylase has an optimum pH and temperature of 5 and 40oC, respectively. The activity of α-amylase was increased by NaCl and KCl and inhibited by other compounds such as MgCl2, CaCl2, Urea, EDTA, and SDS. Native-PAGE electrophoresis of α-amylase showed two isoenzymes, one major and one minor band showing α-amylase importance in the carbohydrate metabolism of the insect.

Keywords:*Rhynchophorus ferrugineus, Native-PAGE electrophoresis, α-amylase, digestive physiology*

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PS2M235

Physiology P2

Induced transcription profile of cecropin and defensin gene families in vector mosquito, *Armigeres subalbatus*

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Cecropins and defensins, well-known antimicrobial peptide gene families, are related to innate immunity in vertebrate and invertebrate. Five cecropin genes, AsCecA1, AsCecA2, AsCecB1, AsCecB2 and AsCecN, and four defensin genes, AsDefA1, AsDefA2, AsDefB and AsDefC, were cloned from Armigeres subalbatus. To understand the induced temporal transcription profiles of cecropin and defensin gene families in Ar. subalbatus, mosquitoes were injected with heat-killed bacteria, Escherichia coli BL21 and Staphylococcus aureus CCRC 15211, respectively, and examined the transcriptional level of cecropin and defensin genes from 10 min to 120 h after treatment with real-time PCR. The results revealed that higher transcriptional level was induced in virgin female mosquitoes treated with heat-killed E. coli than S. aureus. In addition, the AsCecB2 was the only exception that no obvious induction among the cecropin and defensin genes tested. Induced temporal transcription profiles for treatments of E. coli and S. aureus could be divided into three types shown as follows: first, up-regulation at the same time, i.e., AsCecA2, AsCecB1, AsCecN, AsDefA1 and AsDefB; second, up-regulation at different time, i.e., AsDefA2; third, irregular induced transcription profile, i.e., AsCecA1. As mentioned in above, transcriptional regulation of antimicrobial peptide gene families is complicated and further investigation in the future will be needed.

Keywords:*cecropin, defensin, Armigeres subalbatus*

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Physiology

P2

Temperature dependent changes of chemical components in overwintering and non-overwintering predatory ladybird *Hippodamia variegata* (Coleoptera: Coccinellidae)

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Hippodamia variegata (Goeze) is a widespread aphidophagous predator that has been reported as the most important natural enemy of aphids, in many countries. Coccinellids, as many other insect species, minimize low temperature injuries by a series of behavioral and physiological strategies such as seeking shelter, dormancy and accumulating sugars and polyols. Adults of H. variegata cease their reproduction around the middle of October and spend autumn, winter and early spring in altitude of 1800-2200m in aggregations beyond the tore bushy plants. In this study, biochemical parameters were measured in overwintering and non-overwintering adults of H. variegata collected from mountain of Hamadan, Iran and farm from September 2010 to June 2011. The concentrations of cryoprotectants (myo-inositol, trehalose, glycerol and glucose) fluctuated during the study period. Overwintering coccinellids displayed 45 folds higher myo-inositol accumulations compared to non-overwintering insects. However, trehalose, glycerol and glucose contents increased 3 to 6 folds. It was suggested that myo-inositol is the most important cryoprotectants in this species. Large amounts of metabolic reserves in the form of glycogen accumulated prior to overwintering. Glycogen began breakdown and polyols accumulated when ambient temperature decreased from October to December. In the other hands, there was a significant correlation between ambient temperature and glycogen decrease, and myo-inositol, sugar and polyols increase. Findings indicated that low temperatures may induce polyols accumulation in H. variegata.

Keywords:*Overwintering, Hippodamia variegata, Cryoprotectants, Polyols, Diapause*

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Physiology P2

The effect of four different host plants on digestive proteolytic and amylolytic activity of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae)

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The cotton bollworm, Helicoverpa armigera (Hubner) is an important and polyphagous pest worldwide. Digestive proteolytic and amylolytic activity of the larvae of H. armigera fed on different host plants including white kidney bean, (cultivar Dehghan), common bean (cultivar Khomein), red kidney bean (cultivar Goli) and soybean (Williams) was determined. The substrates Azocasein (pH 6 to 12) and starch (pH 2 to 10) were used for assaying proteolytic and amylolytic activity of midgut extracts from H. armigera. The experiments were preformed at 25±1ºC, 65±5% RH and a photoperiod of 16:8 (L: D) h. The results of this study showed that the highest general proteolytic activity of the third, fourth and fifth instar larvae was on Goli (8.89 U/mg), Dehghan (9.08 U/mg) and Dehghan (8.85 U/mg), respectively. In addition, the highest amylolytic activity of the third, fourth and fifth instar larvae was on Goli, Goli and Khomein (0.873, 0.998 and 0.899 U/mg, respectively). Optimum pH of digestive proteolytic and amylolytic activity in the larvae fed on different host plants was observed on Williams (0.011U/mg) probably due to the presence of serine protease inhibitors in this cultivar, resulting in hyperproduryction of chymotrypsin ans tryptic on Williames (0.370 ± 0.03 and 0.303 ±0.03 U/mg, respectively) , in response to the inhibition of the serine proteases. The results indicated that the Dehghan and Williams cultivars were the most suitable and unsuitable hosts to this pests, respectively.

Keywords:*cotton boll worm, Helicoverpa armigera, digestive enzymes, host plants.*

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PS2M238

Physiology

P2

Digestive proteolytic and amylolytic activity of *Helicoverpa armigera* (Lepidoptera : Noctuidae) in response to time, different instars of larvae and cultivar

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The cotton boll worm, Helicoverpa armigera (Hubner) is a serious pest of different agriculture crops in most parts of the world.The best time for the activity of protease and amylase in the midgut of the larvae of H.armigera was determined at 25±1ºC, 65±5%RH and a photoperiod of 16:8 (L: D) h. The midgut from H.armigera fifth instar larvae reared on chickpea (cultivar Arman) were anatomized and the supernatants were used as the source of enzyme. The substrates of Azocasein and Starch were used for measuring proteolytic and amilolytic activities, respectively. The activity of protease enzymes increased linearly for 20 to 60 minutes from the incubation time. But at the incubation time, it turned 80 minutes toward the linear mode. In addition, the activity of amylase enzyme increased linearly for 20 to 40 minutes from the incubation time. But at the incubation time, it turned 60 minutes toward the linear mode. The results showed that the means of linear part of the absorb time reaction is the appropriate time for the examination of the proteolytic and amilolytic activites. Also, significant relationship between the age of insect and variety on general proteolytic and amylolytic was determined by factorial design between two treatments. The results showed that larval instars and variety have significant effect on general proteolytic and amylolytic activites ($P< 0.01$).

Keywords:*Helicoverpa armigera, different time, different instars of larvae, host plant variety, digestive*

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Physiology

P2

Effect of different host plants on some biochemical characteristics of digestive proteases of *Hellicoverpa armigera* (Lepidoptera: Noctudidae)

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The cotton bollworm, Helicoverpa armigera (Hubner) is one of the serious pests of different economic crops in many parts of the world including Iran. Study of insect digestive enzymes is necessary in order to reach practical and new pest management technologies. The midgut from H. armigera fifth instar larvae reared on artificial diets based on different host plants including common bean (cultivar Khomein), white kidney bean (cultivar Dehghan), red kidney bean (cultivar Goli) and soybean (cultivar Williams) were anatomized, and the supernatants were used as the source of enzyme. The results of zymogram analysis on gel showed that protease enzymes in midgut of the cotton bollworm fed on different host plants had two isoforms. The km valves of 7.830 and 5.390 mm and the Vmax valves of 0.033 and o.016 mmol/min were estimated for trpsin on Williams and Goli, respectively. Also calculated km and Vmax for chymotryipsin were 7.930 and 3.260 mM and 0/057 and 0.028 mmo1/min, respectively. Determining biochemical characteristics of insect digestive enzymes could be useful to reach safe and suitable controlling methods, as well as, to obtain better understanding of the enzymes role in nutritional physiology of the insect pests.

Keywords:*Helicoverpa armigera, kinetic, zymogram,host plants*

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Physiology

P2

Identification and characterization of α-amylase in the iranian population of the Rose Aphid, *Macrosiphum rosae* (Linnaeus, 1758) (Hemiptera: Aphididae)

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Aphids are considered a serious agricultural and horticultural pest that causes serious damage to crops by their feeding, and, more importantly, by transmitting various plant viruses to their hosts. In addition, 'honeydew' excreted by the aphids attracts saprophytic fungi which cover the leaves leading to reduction of photosynthetic capacity of the host plant. Rose aphid, M. rosae (Hemiptera: Aphididae), is a cosmopolitan pest that cause severe damage to various members of the Rosaceae. This pest may have various hosts during the year, however, it is most commonly found feeding on roses. The aim of this study was extraction and characterization of α-amylases of M. rosae to get a better understanding of the digestive physiology of this insect species. First one population of aphid was reared on host species. After preparing aphids with the same age, adult insects were collected for further testing. Adult insect (200 numbers) were homogenized and their enzymes were extracted. The activity of digestive α-amylase suing 1% starch as a substrate was determined to be 1.59± 0.0051 Unit. Optimum pH was determined to be 7.0. Optimum temperature was 45oC but the enzyme was active in a wide range of temperature from 20 to 60oC. Zymogram analysis using vertical gel electrophoresis showed that only one amylase band is present in the gel. Thus, these experiments showed that Rose aphid has optimum activity at neutral pH and has only one isozyme.

Keywords:*Native-PAGE electrophoresis, α-amylase, digestive physiology*

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Physiology

P2

Evaluation of proteolytic activity in *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) (Olivier, 1790) and efficiency of synthetic inhibitors in electrophoresis

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Rhynchophorus ferrugineus (Coleoptera: Curculionidae) is an important pest of dates in Iran and Persian Gulf countries. This pest has been observed in Iran for the first time in 1992 from a village in Saravan (Sistan and Baluchestan province) and then its damage has been observed in different places. Thus, the aim of the current study was identification and characterization of the proteolytic activity using general and specific substrates to obtain a better understanding of digestion physiology in the pest. Therefore, the enzymes in the digestive tract of the larvae were isolated and its properties were investigated using the general substrate (Azocasein) and specific substrates of serine proteases. Specific used substrates were Nα-benzoyl-DL-arginine p-nitroanilide (BApNA) for trypsin activity and N-succinyl-alanine-alanine-proline-phenylalanin p-nitroanilidine (SAAPFpNA) for chymotrypsin activity. This study showed that the optimum proteolytic pH activity by using Azocasein substrate increased in both at pH 6 and 10 indicating the existence of two series of enzymes digesting protein (serine and cysteine) in the digestive system of the insect. Further studies using specific substrates at three pHs of 6.0, 8.0 and 10.0 showed that activity of chymotrypsin is more than trypsin and the enzymes activity were increasing in higher pHs in both enzymes. The effect of synthetic inhibitors TLCK, TPCK, PMSF, E64 and SBTI on the proteolytic activity in SDS-Electrophoresis showed that a mixture of the proteolytic enzymes are present in the insect gut.

Keywords:*Native-PAGE electrophoresis, proteolytic activity, digestive physiology*

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PS2M242

Physiology

P2

Characterization of α-amylase in the Italian Locust, *Calliptamus italicus* (Linnaeus 1758), (Orthoptera: Acrididae)

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Calliptamus italicus L. is a polyphagous insect which is widely distributed throughout of Europe, Middle East and North Africa. Unlike the Moroccan and Desert locusts, C. italicus migrate short distances. In some years, due to changes in weather conditions and dry vegetation, they make damage to the adjacent field crops. Aim of the current study was to investigate α-amylase activity of this insect species. Insects were collected from Tehran province and 10 adult insects were homogenized and their enzymes were extracted. The activity of digestive α-amylase was 0.022± 0.0081 Unit activity. Italian Locust α-amylase activity pH was determined to be around 8.0. The enzyme activity was decreased sharply after pH 9.0, but the enzyme activity steadily increased from pH 3.0 to 9.0. Thus it could be said that the enzyme is more active in acidic region than the alkaline condition. Generally, optimal pH is corresponding to the pH prevailing in the midgut from which the enzyme has been extracted so these discrepancies seen in midgut pH are related to the different feeding habits and feeding sources. The optimal temperature for C. italicus α-amylase activity was 40°C, the enzyme was active over a broad temperature range from 30 to 60°C. Zymogram analysis of this grasshopper by vertical slab gel electrophoresis using starch as substrate showed 9.0 bands of amylase activity indicating extensive amylase activity.

Keywords:*Native-PAGE electrophoresis, α-amylase, digestive physiology*

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Physiology

P2

Identification and characterization of α-amylase in citrus swallowtail, *Papilio demoleus* (Linnaeus 1758), (Lepidoptera: Papilionidae)

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Papilio demoleus L., commonly known as the lime or Citrus Swallowtail that has been distributed in many asian countries. It insect pest has a good potential to be a major pest in India, Pakistan, Iraq and the other Middle East countries. Thus, the aim of the current studies was to investigate α-amylase activity of the insect. So, the last instar larvae were collected from around Surry (Mazandaran Province). Then larvae placed on ice to become motionless and their guts were isolated. After that larvae gut were homogenized in distilled water and centrifuged and supernatant was kept at -20 oC as an enzyme source. This study showed that α-amylase activity was present in the insect larvae in order to digest carbohydrates. Optimum temperature and pH of the α-amylase using starch as substrate were determined to be 45 oC and 8.0, respectively. Enzyme kinetic studies using five different substrate concentrations including 0.05, 0.1, 0.2, 0.4, and 1.0 % and fixed enzyme concentration showed that Vmax and Km were 0.19 U and 0.46 % substrate, respectively. Understanding of the digestive physiology and α-amylase activity of Citrus Swallowtail is important when new management strategies for this economically important pest are devised.

Keywords:*Native-PAGE electrophoresis, α-amylase, digestive physiology*

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Physiology P2

Ecological significance of cooling rates improving the cold hardening of overwintering *Hippodamia variegata* (Coleoptera: Coccinellidae)

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Hippodamia variegata (Goeze) is an aphid predator used in the biological control of plant lice in cereals and oil plants in diverse countries. Low temperature is the major adverse environmental factor that insects must overcome in temperate and cold regions. Therefore, cold hardiness strategy of overwintering H. variegata, collected from mountains of Hamadan, Iran was studied from October 2010 to April 2011. In order to assess the acute freezing tolerance status of the adult insects, whole body supercooling point (SCP) and the ability to survive at subzero temperatures with two cooling rates of 0.5 and 0.04 °C/min was investigated in each month. This study showed that H.variegata exhibited high SCP with mean seasonal values upper than -10°C. Coccinellids were alive below SCP. The lower lethal temperatures (LLTs) of overwintering adults were approximately 10 and 20°C lower than their SCPs at cooling rates of 0.5 and 0.04 °C/min, respectively, indicating that this species is would be a freezing tolerant insect. These findings suggests ecological importance of low cooling rates as occur naturally in the overwintering sites and enhance the development of cold hardiness. As LLTs of overwintering H. variegata were about 26-45 °C lower than average ambient temperatures, it seems they successfully pass the severe condition of winter in Iran.

Keywords:*cold hardiness, Hippodamia variegata, cooling rate, soopercooling point*

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PS3M245

Ecology

P3

The architecture of Sunggau in Bangka Island, Indonesia - An artificial wild honey bee nest

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Business of honey bee has not positively responded by the people of Bangka Island, even though the production of honey is from forest. The ideal architecture of artificial bee nest (sunggau) needs to be identified in order to increase the successfulness of the forest honey bee to build their nests, and to gain the utmost volume of honey. The sunggau architecture of wild bee (Apis dorsata) in Bangka consists of a piece of log, its average length is 344 cm, diameter 28 cm, front height is 213 cm, rear height is 119 cm, it forms an angle of 19°, opening width is 78 cm, the width of the middle part is 118 cm, the width of the back part is 79 cm, the length of inner area is 181 cm, and its volume is 2.9 m^3. The main log should be dry and / or fresh and it should not be decayed or moldy. Along the edge and the rear of the sunggau are covered by shrub/bush, its diameter +/- 5 cm or by placing pieces of logs, diameter 4 - 6 cm. The directions of the opening of the sunggau can be any, but need a space free from shrub and trees to be the direction of the bees coming in. The frequency of the honey harvest has stronger correlation with angle of sunggau and room volume. Direction and type of wood have no strong correlation.

Keywords:*architecture, sunggau, Apis dorsata, wild honey bee, artificial nest, Bangka Island*

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PS3M246

Ecology

P3

Behavioural and physiological differences between the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the Natal fruit fly, *C. rosa* Karsch and how it relates to their distribution

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Ceratitis capitata and C. rosa are serious pests of fruit produced in South Africa. Being of phytosanitary concern, they restrict international fruit trade. A study was conducted to determine how behavioural and physiological differences between these species relate to their South African distribution. Field sampling sites were chosen to represent different climatic regions. At each site, three yellow bucket traps, baited with Biolure® Fruit Fly and dichlorvos to kill the attracted flies, were placed in host trees in back gardens and inspected monthly for two years. Ceratitis rosa adults and pupae are more sensitive to desiccation and pupae less sensitive to immersion in water than C. capitata, suggesting that C. capitata may be better adapted to drier climates, while C. rosa may be better suited to tropical conditions. This was reflected in their South African distribution where C. capitata was widespread, while C. rosa was absent from or less abundant in the drier regions where C. capitata had a high abundance. Along the wetter, moderate south and east coast, C. rosa was the most abundant species. Ceratitis rosa has a crepuscular behaviour and cannot mate if temperatures during the sunset period fall below 17°C. In addition, C. capitata can develop rapid cold hardening after low temperature exposure faster than C. rosa and the response lasts longer, suggesting that C. capitata has an advantage over C. rosa for survival in colder climates. The data suggests that globally, C. rosa does not have the potential to become as widespread as C. capitata.

Keywords:*South Africa, relative abundance, phytosanitary*

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Ecology

P3

Physical and chemical stimuli that affects *Dactylopius opuntiae* anchorage

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The "grana insect complex" is formed by two different species (Dactylopius coccus and D. opuntiae) and it is important in mexican agriculture. D. cocous or "grana fina" is cultivated on nopal (Opuntia ficus.indica) to obtain carminic acid an important mexican export commodity. Nopal plants infested by "grana fina" may live up to 6 or 7 years while infested by D. opuntiae or "grana silvestre" may die in 6 months. The latest also produces carminic acid, but its yield is negligible. Both insects may live in the same region, so producers are aware of the potential invasion. "Grana silvestre" is controlled with chemical pesticides. Little is known about the stimuli involved in host selection and even more, the clues used by nymphs to settle down. Once the insect settles, it won´t move anymore. We tested different physical and chemical treatments to reduce the number of nymphs on the nopal cladodes. Nymphs do not discriminate between basic colors nor lines or dots presented on a white floor. However, nymphs discriminate between basic colors and horizontal lines and dots and a black wall when these stimuli were presented on a wall. Texture is not relevant for nymph anchorage. 50-d after application of an alcoholic dilution of cineol, eugenol, menthol and alcoholic extract of "grana silvestre" the number of nymphs on the cladode was reduced 71, 67 and 63% compared with the control. 107 d after application, cladodes treated with menthol and cineol had 75.7 and 35.45% (respectively) less colonized area than the control.

Keywords:*nopal, carminic acid, cochineal scale*

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Ecology
P3

Effect of weight and age at mating on *Copitarsia decolora* reproductive potential

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The cabbage moth, Copitarsia decolora is an important pest of cruciferous plants in Mexico and it is a quarantine insect for USA. A single larvae may destroy a whole plant. The use of mating disruption tactics requires knowledge on the mating system of the target insect. This abstract reports our findings on the effect of bodysize and age at mating on reproductive performance of this pest. Fecundity and fertility was affected by female and male age at mating and the female weight. Four-d-old females laid significantly more eggs than any other age tested. Females that mated with 2-4-d-old males laid significantly more eggs than those that mated 6-8-d-old males. Similarly, females mated to 2-4-d-old males produced more fertile eggs than those mated to 6-8-d-old-males. The total number of oviposited and fertile eggs/day/female followed a similar trend. Oviposition dynamics were clearly different. Four-d-old females mated to 2-4-d-old males oviposited more than 80% of their total egg load by day 6, while 4-d-old females mated to 6-8-d-old reached similar figure on the 10th day. However, more than 80% of fertile eggs of 4-d-old females mated to 6-8 d-old were laid by day 12. Female fecundity was significantly and positively affected by female weight. Moreover, male weight significantly affected female fertility. Females mated at early age lived less than 8- and 10-d-old females. Female and male weight did not affect insect longevity. Based on our results, mating disruption or any other behavioral control method may be used in an IPM scheme.

Keywords:*bodysize, fecundity, longevity*

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Ecology

P3

Impact of leaf-miner insects on the primary metabolism of their host-plant: Manipulating from the inside

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Endophytophagous insects, such as stem-boring, gall-forming and leaf-mining insects, live within plant tissues and feed internally. This life-style presumably provides adaptive advantages for the insect over other external-feeding modes by allowing access to most nutritional tissues while avoiding main plant defensive compounds. This selective-feeding behavior can be reinforced by manipulating the plant physiology, as suggested by the formation of 'green-islands' around mining caterpillars in yellow leaves. We study this so-called 'nutrition hypothesis' on the system Malus domestica/Phyllonorycter blancardella. Thanks to colorimetric assays, capillary electrophoresis and GC/MS, we quantified proteins, starch, total soluble carbohydrates, main individual carbohydrates and free amino acids on green and yellow mined leaves. Our results suggest an active manipulation of the plant primary metabolism by the leaf-miners with a plant response to insect herbivory strongly dependent of the feeding mode of the larva. Indeed, this species is characterized by a larval hyper-metamorphosis with first instars being fluid-feeders and last instars being tissue-feeders. Tissue-feeding larvae control their micro-environment in order to generate a microenvironment with all the nutrient supply needed for their growth and survival but fluid-feeding larvae seem to be limited in such capacity to manipulate the plant physiology. All together the observed plant metabolic changes in the mine further strengthen the hypotheses that mines behave independently from the hosting leaf, operating a metabolic machinery on their own. By isolating the mine from the rest of the leaf, the insect buffers environmental variations of plant quality, presumably protects itself from plant defenses and obtain a nutritional autonomy.

Keywords:*Leaf-miner microlepidoptera, physiological manipulation, green-islands, Wolbachia, nutrients*

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Ecology
P3

Cooperation between *Wolbachia* and leaf-miners to produce "green-islands": Insights into the plant-insect-bacteria interaction

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The life-cycles of many organisms are constrained by the seasonality of resources. This is particularly true for leaf-mining herbivorous insects who use deciduous leaves to fuel growth and reproduction even beyond leaf fall. Our results suggest that an intimate association with bacterial endosymbionts might be their way of coping with nutritional constraints to ensure successful development in an otherwise senescent environment. We show that the phytophagous leaf-mining moth Phyllonorycter blancardella (Lepidoptera) relies on bacterial endosymbionts, most likely Wolbachia, to manipulate the physiology of its host plant resulting in the "green-island" phenotype - photosynthetically active green patches in otherwise senescent leaves - and to increase its fitness. Curing leaf-miners of their symbiotic partner resulted in the absence of green-island formation on leaves, increased compensatory larval feeding, and higher insect mortality. Our results suggest that bacteria impact green-island induction through manipulation of cytokinin levels. All individuals analyzed so far are closely associated with Wolbachia and a key enzyme of the cytokinin biosynthetic pathway (ipt: isopentenyl transferase) has been isolated, cloned and sequenced from the Wolbachia genome (tRNA-ipt Wo). This key enzyme is clearly expressed in Wolbachia-infected insects. This is the first example of an insect bacterial endosymbionts associated with alterations of the plant physiology. Our results highlight (i) the intimate interactions between the bacterial symbionts and their insect host and (ii) the presence of cytokinin-producing Wolbachia and associated plant physiological alterations in different population of this species as well as in different plant-leafminer systems.

Keywords:*Wolbachia, leaf-miner, green-island, cytokinins, Insect plant-interaction*

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Ecology

P3

The role of *Atelocera raptorial* (Hemiptera : Pentatomidae) in the succession of fungal populations within pecan nut (*Carya Illinoensis*) orchards in South Africa

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[1]University of the Free State, South Africa, [2]University of the Free State, South Africa, [3]University of the Free State, South Africa

Insects harbour and disseminate fungal propagules and may contribute to the occurrence and development of disease within crop production, consequently affecting the yield in terms of quantity, quality and safety. The role of insects in the spread and occurrence of fungal pathogens is not well documented for most crop production systems and no such data exists for the dissemination of fungal pathogens within new crops, e.g. pecan nuts, in South-Africa. The main aim of the study is to follow the succession of the fungi associated with Atelocera raptoria during the seasonal production of pecan nuts; secondly, to compare the occurrence and possible sources of fungal pathogens in different niches such as soil, air and the phylosphere with the fungi associated with A. raptoria. Sampling was conducted at the Vaalharts district at three different times during the growing season of the pecan nuts. One hundred pentatomid specimens, as well as air, soil and leaf samples were taken on three different occasions. Preliminary results indicated that potential pathogens and contaminants from genera such as Alternaria, Aspergillus, Cladosporium, Fusarium and Penicillium were prominent on A. raptoria and in the environment. This study provides relevant information emphasizing the dynamics of insect-fungal associations in nut orchards. Furthermore, it is envisaged that insect-fungal relations could likely be incorporated into a bio-monitoring protocol for fungal communities.

Keywords:*Atelocera raptoria, pecan nuts, fungal pathogens, dissemination, succession, bio-monitoring*

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Ecology

P3

Greenhouse pests activity patterns

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Annual rhythms of several greenhouse pests in greenhouse pot plant cultures are described over a ten year period period. Circadian rhythms of thrips and a lepidopteran pest are described. Temporal patterns of behaviour in greenhouses are discussed and implications for pest control are indicated.

Keywords:*annual rhythm, circadian rhythm, Frankliniella occidentalis, Sufetula, Orthomorpha, Duponchelia*

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PS3M253

Ecology

P3

Effect of different environmental conditions on plant defenses mediating interactions between the Mountain Pine Beetle and its host pine trees

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The Mountain Pine Beetle (Dendroctonus ponderosae) has killed millions of lodgepole pine (Pinus contorta; historical host) trees in British Columbia, Canada and has moved eastwardly into the hybrid zone of lodgepole x jack pine (Pinus banksiana) and more recently into jack pine in Alberta. Our objectives were (1) to develop a chemical profile of volatile organic compounds (VOCs) released from the host trees (P. contorta, P. banksiana); (2) to evaluate if VOC profiles vary with different environmental (ambient vs. water deficit) and biological treatments (fungal inoculation with G. clavigera vs. control). Fungal-inoculated trees release more overall monoterpenes than control trees. Lodgepole pine emitted higher amounts of monoterpenes than jack pine. The effect of water regime on overall volatile emission seems to be species or site specific. Lodgepole and jack pine trees that experienced ambient water conditions emitted more and less total VOCs, respectively, than trees subjected to water deficit for each species. Chemical analysis of the phloem is in process to evaluate the impact of environmental and biological treatments on the chemically-mediated interactions between MPB and its host trees.

Keywords:*volatile organic compounds, mountain pine beetle, plant-insect interactions*

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Ecology

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Comparisons of the parasitism by four indigenous *Trichogramma* species on the rice striped stem borer, *Chilo suppressalis* at different temperature and relative humidity regimes

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The striped stem borer, Chilo suppressalis (Walker), is one of the most economically important rice pests worldwide. However, biological control of this pest using natural-enemy insects has been rarely documented to date. Indigenous natural populations of four Trichogramma species (i.e., T. japonicum, T. chilonis, T. dendrolimi and T. ostriniae) were collected from the eggs of C. suppressalis in paddy field. With the objective of screening suitable candidate species for controlling the striped stem borers, the parasitism of these four Trichogramma species on their native host eggs, C. suppressalis, was evaluated at five temperatures (18, 22, 26, 30 and 34℃) and four relative humidity regimes (30, 50, 70 and 90%). The results indicated temperature and humidity significantly affected the parasitism of all species. Trichogramma chilonis had higher parasistim at 26 ℃ than at other temperature regimes. In contrast, T. dendrolimi, T. ostriniae as well as T. japonicum performed better within a wider-adapted temperature range. All four Trichogramma species parasitized the largest number of C. suppressalis eggs at 70% RH. Parasitism differed significantly among Trichogramma species at all temperatures tested with an exception of 30 ℃and all relative humidity regimes with an exception of 50% RH. Generally, T. dendrolimi and T. japonicum apparently performed better than the other species at 18-26 and 30-34℃, respectively. Whereas, T. ostriniae parasitized the least number of host eggs at 30-70% RH. Both T. dendrolimi and T. japonicum appear to be promising candidates for augmentative release against C. suppressalis in northeastern China.

Keywords:*Trichogramma, Temperature, Humidity, Performance, Biological control*

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Ecology

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Nutritional variations affect the larval behaviour of the spruce budworm (*Choristoneura fumiferana* (Clem.).

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The pressure of insect outbreaks can alter the allocation of nutrients in host plants. This re-allocation can directly affect the insect's performance. Low nutritional quality can promote migration to look for a better food resource. To determine whether a nutritional stress effects biological performance and as a consequence feeding behavior, we observed the feeding behavior of 50 larvae of 6th instar spruce budworm (Choristoneura fumiferana Clem.) (Lepidoptera: Tortricidae) during four hours feeding periods. Larvae were reared on two types of artificial diet, a nutritionally-balanced diet and a less suitable diet. The results show that nutritional stress affects behavior and the biological performance of females. Individuals have short periods of feeding and probing, with these behaviors being longer in the second generation. This study suggests that the low quality of foliage induced by long periods of defoliation may cause an active behaviour change in insects feeding on this foliage.

Keywords:*spruce budworm, larval behaviour*

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Ecology

P3

Allopatric distribution and diversification without changing diet in a bryophyte-feeding basal moth lineage (Lepidoptera: Micropterigidae)

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The Lepidoptera represents one of the most successful radiations of plant-feeding insects, which predominantly took place within angiosperms beginning in the Cretaceous period. Angiosperm colonization is thought to underlie the evolutionary success of the Lepidoptera because angiosperms provide an enormous range of niches for ecological speciation to take place. By contrast, the basal lepidopteran lineage, Micropterigidae, remained unassociated with angiosperms since the Jurassic but nevertheless achieved a modest diversity in the Japanese Archipelago. We explored the causes and processes of diversi?cation of the Japanese micropterigid moths by performing molecular phylogenetic analysis and extensive ecological surveying. Phylogenetic analysis recovered a monophyletic group of approximately 25 East Asian endemic species that feed on the liverwort Conocephalum conicum, suggesting that niche shifts hardly played a role in their diversi?cation. Consistent with the weak ?ying ability of micropterigid moths, the distributions of the Conocephalum feeders are each localized and allopatric, indicating that speciation by geographical isolation has been the major process shaping the diversity of Japanese Micropterigidae. To our knowledge, this is the largest example of radiation of herbivorous insects that does not accompany any apparent host-plant differentiation.

Keywords:*ecological speciation, molecular dating*

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Ecology

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Determination of the maternal effect of *Aspidiotus nerii* Bouche (Hemiptera: Diaspididae) by using life table

ismail Karaca[1], Senay Ozger[2], Alime Bayındır[3], Mehmet Sedat Sevinc[4]

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Aspidiotus nerii Bouche (Hemiptera: Diaspididae) is an excellent host for rearing biological control agents for the control of scale insects. This study investigated the maternal effect on the reproduction and development of A. nerii Crawlers at the 1st, 15th and 30th days were transferred to separate clean potato tubers. After the crawlers settled, each potato was divided into cells of 4 cm2 surface area surrounded by 'Stickem Special' to prevent their escape. When all nymphs in the cell became adults, one female and all the males were left in the cells, and the rest of the females were removed. Experiments were done at 25±0.5 °C, 16:8 hours and 65±1% humidity in a climate chamber. Cells were observed daily; nymphs were removed after counting. Variance analysis and Duncan's multiple range test were applied for statistical analysis. The average number of nymphs produced by 1, 15 and 30 day old nymphs were 208.57, 47.82 and 20.07, respectively. Life table parameters were also calculated. Net reproductive rate (Ro), intrinsic rate of increase (rm) and mean generation time (To) were found to be 104.286, 23.911 and 7.525 females/females/generation, 0.109, 0.078 and 0.057 females/females/day, 42.714, 40.616 and 35.439 days, respectively.

Keywords:*Maternal effect, Aspidiotus nerii, intrinsic rate of increase, net reproductive rate*

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Ecology

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Life Tables Variability: with a mathematical discussion of the application of the jackknife technique to the estimation of the variance of net reproductive rate

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Life tables were constructed for Bactrocera cucurbitae on Cucumis sativus L. under simulated field conditions. Eggs were artificially introduced into cucumbers and kept at 25ºC in a growth chamber, and the values of population parameters were measured for two replicates. The intrinsic rates of increase (r) found for the two replicates were 0.1354 and 0.1002 day-1, the net reproductive rates (R0) were 206.3 and 66.0 offspring, and the mean generation times (T) were 39.5 and 42.6 days, respectively. Moreover, for cucumbers kept in the field and covered with leaves, the population parameters (r, R0 and T) for two replicates were 0.0935 and 0.0909 day-1, 17.5 and 11.4 offspring, and 34.0 and 35.0 days, respectively. However, if cucumbers were kept in the field but were not covered, the population parameters (r, R0 and T) for two replicates were 0.1043 and 0.0904 day-1, 27.7 and 10.1 offspring, and 32.8 and 27.2 days, respectively. These results showed that life tables constructed in the laboratory can differ significantly from life tables constructed using field data. Moreover, our results revealed significant variability between replicates. The differences between life tables and the variability between replicates found by this study must be considered in practical applications of life tables in pest management. The mathematical analysis shows that the jackknife technique generates biologically meaningless values of net reproductive rate and consequently results in overestimation of its variance. We suggest that the jackknife technique should not be used for the estimation of variance of the net reproductive rate.

Keywords:*Bactrocera cucurbitae, Cucumis sativus, jackknife method, life table*

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Ecology

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Effect of host plant on feeding, biological and morphological parameters of *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae)

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Liriomyza huidobrensis (Blanchard) is a quarantine key pest to the horticultural sector in East Africa. To understand its behavior for formulation of suitable management strategy, a study was conducted to determine the effect of host plant on its performance. Vicia faba L., Phaseolus vulgaris L., Pisum sativum L. and Lycopersicon esculentum M. were evaluated for their suitability for feeding, oviposition, survival and development of L. huidobrensis under both no-choice and choice conditions, simulating monoculture and intercrop systems, respectively. Lycopersicon esculentum was the preferred host with mean feeding punctures/cm2 of 23.34±3.35 and 11.59±1.36 and mean number of eggs/ cm2 of 0.57±0.11 and 0.08±0.04 for the no-choice and choice conditions, respectively. It was however the least suitable for development of L. huidobrensis, with the lowest number of pupae and adults (2.50±0.73 and 1.38±0.46 respectively), survival rate of 6.05±1.95% and fitness as measured by female and male wing length of 1.84±0.04mm and 1.57±0.03mm, respectively. This makes it a good candidate trap crop for management of this pest. Vicia faba, on the other hand, was the most suitable for development of the pest (42.88±11.80 pupae and 32.13±8.77 adults), survival (65.01±13.86%) and fitness (2.18±0.03mm and 1.77±0.04mm), making it a suitable host for mass rearing of L. huidobrensis. Previous experience (rearing history) of the pest significantly affected the selection for feeding and egg laying, but did not affect host suitability for development. Liriomyza huidobrensis fed and oviposited significantly more under no-choice condition than choice set-up suggesting the potential of mixed cropping in controlling L. huidobrensis infestations.

Keywords:*Agromyzid, Liriomyza huidobrensis, host plant, feeding, oviposition, development, Survival*

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Ecology
P3

Population dynamics and 'outbreaks' of Diamondback moth, *Plutella xylostella*, inGuangdong province, China: Climate or the failure of management?

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Diamondback moth, or DBM, became the major pest of brassica vegetable production in Guangdong, a province in south-eastern China, in the late 1980's and has continued to challenge growers, particularly during the spring and autumn. Control has relied on insecticides and, as has happened in other parts of the world, resistance to these has evolved and subsequent field control failures have occurred. We review and summarize the history of DBM management in Guangdong. We show that the geographic distribution of the pest in China is well described by a simple climate niche model. Our model predicts the seasonal phenology and some of the variation in abundance amongst years in Guangdong. Discrepancies may reflect migration and insecticide use at a landscape level. The scale of the pest problem experienced varies with management practices. Local production breaks, and strict post harvest hygiene are associated with lower pest pressure on large scale production units. As more and more insecticides become ineffective the need to implement an IRM (Insecticide Resistance Management) strategy, as well as basic IPM (Integrated Pest Management) practices, will become more pressing. The potential use and development of a better forecasting system for DBM that will assist these 30 developments is outlined.

Keywords:*bioclimatic modelling, population outbreaks, Plutella xylostella, resistance, IPM*

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Ecology

P3

Effects of cold storage on survival, predation and reproduction of pre-wintering *Harmonia axyridis* (Pall)

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The multicolored Asian ladybird beetle, Harmonia axyridis (Pallas), is considered an important generalist predator against aphids, mealybugs, scale insects and spider mites. There are currently abundant natural resources of overwintering H. axyridis in Asia and North America. The availability of an adequate cold storage method would enable the use of field-collected pre-wintering ladybirds for pest suppression in the following season. We studied the effect of cold storage (30, 60, 90, 120 and 150 days stored at -3, 0, 3 and 6 ℃) on survival, fecundity and predation in field-collected populations. The survival of both female and male ladybirds decreased significantly as storage duration increased at -3 and 0 ℃. The ladybirds showed more than 80% survival when they were stored for 150 days at 3 and 6 ℃. Long-term cold storage had different effects on the fecundity of H. axyridis at different temperatures. Prolonged cold storage at both 3 and 6 ℃ shortened their pre-oviposition duration and had no adverse effect on reproductive capacity as compared to that of unstored individuals. The adults that experienced 90-day storage at 0 ℃ had the shortest pre-oviposition duration and the largest reproductive capacity. The individuals that were stored for 150 days at 3 ℃ consumed significantly more aphids than the unstored ones. Generally, 3-6 ℃ is a suitable temperature for cold storage of the ladybird without any reduction in fitness. This study will help the exploitation and application of pre-wintering H. axyridis for the biological control of insect pests.

Keywords:*Harlequin ladybird, Lady beetle, Cold tolerance, Biological control*

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Ecology

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Transmission rate of rice stripe virus by *Laodelphax striatellus* from different geographical population

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The small brown planthopper, Laodelphax striatellus (Fallen) can transfer the Rice stripe virus (RSV) to host plants, which then develop rice stripe disease. RSV is a typical member of the genus Tenuivirus, and is transmitted in the circulative/persistent manner by L. striatellus. There are two paths for virus transmission between vectors. One is transovarially transmitted to progeny from infected parents (vertical transmission), and the other is horizontal transmission by sucking infected plants. We examined horizontal, vertical and mixed (including horizontal and vertical) transmission rates of RSV by L. striatellus from one not-epidemic area (Fuyang in Zhejiang province) and three epidemic areas (Yangzhou and Peixian in Jiangsu Province, and Donggang in Liaoning province). The results indicated that RSV acquisition rate of naive L. striatellus from four populations were not significantly different. RSV transmission rate to healthy rice plants by viruliferous L. striatellus in Fuyang population was significantly lower than those in Yangzhou, Donggang and Yangzhou populations. Vertical transmission abilities and mixed transmission abilities of infective L. striatellus in the first generation in tested populations were similar. But in the fourth generation, ratio of RSV-positive offspring individuals from an infective mother in Fuyang population was significantly lower than those in Xuzhou, Yangzhou and Donggang populations. Different transmission abilities of L. striatellus in different populations can have two explanations: either the population genetic structure of RSV was different, or L. striatellus from 4 populations were heterogeneous.

Keywords:*Laodelphax striatellus, Rice stripe virus (RSV), RSV transmission ability, geographical population*

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Ecology

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Effect of temperature on the developmental period, survival and adult longevity of *Sesamia nonagrioides* (Lepidoptera: Noctuidae)

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The effect of temperature on development, longevity and survival of the corn stalk borer Sesamia nonagrioides Lefebvre (Lepidoptera: Noctuidae), has been investigated in the laboratory. The duration of development of egg, 1st, 2nd, 3rd, 4th, 5th larval instar and pupa, as well as adult longevity, at five constant temperatures (14, 17, 21, 25 and 31℃) was measured. Development time decreased significantly with increasing temperature within the range 14-25℃. Survival was higher at medium temperatures (17 to 25℃) in comparison with that at more extreme temperature regimes (14 and 31℃). Duration of all developmental stages differed significantly among various temperatures. Experimental individuals demonstrated low total survival rate (9.3-34.1%) at all temperature regimes. Egg and 1st instar larvae were the stages where S. nonagrioides suffered the highest mortality levels at all temperatures. Thermal requirements of development (developmental thresholds, thermal constant, optimum temperature) were estimated with application of a linear and one non-linear model (Logan I). Upper and lower developmental thresholds ranged between 31.2-36.2℃ and 7.0-10.3℃, respectively. Optimum temperature for development was estimated at between 28.1-30.3℃. The thermal constant for total development was estimated 1000.0 day-degrees. Adult longevity of S. nonagrioides ranged between 6.0 ?17.3 days and decreased with increase in temperature only within the range 14-25℃. Differences between two successive temperatures were significant only between 14 and 21℃. The Weibull distribution was used to describe the age specific survival, which in S. nonagrioides is of "Type I", as the risk of death increases with age.

Keywords:*Sesamia nonagrioides, developmental thresholds, longevity, survival, thermal constant*

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Ecology

P3

Cold adaptation coupled with population expansion during the ice age in the pollinating wasp *Wiebesia pumilae* (Hymenoptera: Agaonidae) of Ficus pumila

Hurng-Yi Wang[1], Chia-Hung Hsieh[2], Chin-Gi Huang[3], Sui-Wah Kong[4], Hsiao-Chi Chang[5], Ho-Huei Lee[6], Shih-Lun Chen[7], Hsy-Yu Tzeng[8], Wen-Jer Wu[9]

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In order to study genetic interactions between host figs and their pollinating wasps and the influence of climatic changes on their genetic structures, we sequenced both cytoplasmic and nuclear genes and genotyped nuclear microsatellite loci from two types of Ficus pumila, the widespread creeping fig and the endemic jelly fig, and from their pollinating wasps, Wiebesia pumilae, found in Taiwan and on nearby offshore islands. Between the two pollinating wasps, the level of divergence in the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene was more than 50-times higher than the two nuclear segments sequenced. In addition, we found an excess of amino-acid substitutions in mtCOI, especially in the lineage leading to jelly-fig wasps. Both nuclear intron and microsatellite loci of jelly-fig wasps showed signs of population expansion beginning during the last glacial age. Finally, jelly-fig wasps lived longer in low (20 °C) than in high (25 and 30 °C) environmental temperatures in survival tests. All of the above results indicate its adaptation to a cold environment, which probably has been facilitated by glacial epochs as the estimation of the time to the most recent common ancestor suggested. Between the two fig plants, a complete segregation of chloroplast DNA was observed, but there was no sign of isolation in nuclear markers, suggesting that the two types of fig trees are still genetically linked. Finally, according to the molecular clock, histories of their local colonization, population demographics, and the formation of current distributions were nicely coupled with past climatic changes.

Keywords:*positive selection, cytochrome c oxidase subunit I, electron transport system*

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Ecology

P3

Transplanting large potted trees: a factor involved in the range expansion of the pine processionary moth, *Thaumetopoea pityocampa*.

Christelle Robinet[1], Charles-Edouard Imbert[2], Jerome Rousselet[3], Daniel Sauvard[4], Jacques Garcia[5], Francis Goussard[6], Alain Roques[7]

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Pine processionary moth, Thaumetopoea pityocampa, is expanding its range northwards and upwards in response to climate warming. The natural spread in latitude was estimated at ca 5.6 km per year during the last 10 years in the southern Paris Basin. However, additional pioneer colonies were recently detected far from the northern edge of the range, and notably near downtown Paris. A study combining several approaches was conducted to determine the origin of these pioneer colonies First, the shortest distance between the pioneer colonies and the previously-infested locations was calculated, and this distance was compared to the female flight distance measured on a flight mill. We found that most of pioneer colonies could not result from natural dispersal. Then, some individuals from the pioneer colonies were genotyped and compared to populations of different areas in France and neighbouring countries. We found that several pioneer colonies had higher probabilities to originate from the southern Mediterranean area than from the northern edge, suggesting a dispersal at very long distance and accidental human transportation. Finally, the presence of natural enemies associated with different life stages of the pine processionary moth was assessed. We found a parasitoid species specific of pine processionary pupae indicating that this natural enemy was probably transported along with moth pupae. These convergent results suggest that pioneer colonies may result from the transportation of large potted trees including pupae in the accompanying soil. Consequently, both global warming and human-mediated dispersal are involved in the range expansion of this insect.

Keywords:*range expansion, climate change, pine processionary moth, Thaumetopoea pityocampa, human-mediated dispersal*

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Ecology
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Predator's non-consumptive effects on herbivores influence herbivore-induced plant responses

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Species change their traits morphologically, physiologically, behaviorally in response to the presence of other species, and these trait changes influence indirectly other interacting species, which is called trait-mediated indirect interaction (TMII). Previous studies have revealed that TMIIs play important role in organization of ecological communities. However, the little is known whether trait change of one species affect the trait changes of other species. To understand the dynamics of TMIIs, we must consider how trait changes interacting with each other. There is increasing appreciation that non-consumptive effects of predators often reduces feeding activity and/or trigger habitat shift of herbivores, which, in turn, increases plant biomass. On the other hand, plants also change their traits in response to the herbivore attack. The magnitude of induced plant responses is largely dependent on patterns of herbivory, such as the amount of damage and position receiving damage. Hence, we hypothesize that predators can alter herbivore-induced plant responses through a behavioral shift of herbivores. We conducted a field experiment to test the hypothesis, using a tri-trophic system of a willow, leaf beetle larvae, and a flower spider. The experiment had four treatments: lethal (tri-trophic with a spider) and risk (tri-trophic with a manipulated predator whose chelicerae were glued), no predator (bi-trophic), and control (plant alone) treatments. We found that spiders' non-consumptive effects suppressed the induced chemical change of willow leaves.

Keywords:*trait-mediated indirect interaction, trophic cascade, non-consumptive effect, herbivore-induced plant responses*

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Ecology

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Inferring the difference of moth fauna composition in subtropical alpine forests - A case study in Hohuanshan Mountain Range, Taiwan

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The moth fauna in Taiwan is continuously explored in these 20 years after the checklist of "Lepidoptera of Taiwan" published in 1992. The efforts of moth study, especially the alpha-taxonomy and hostplant recording, facilitate the subsequent ecological and environmental monitoring using moths as target taxa. Here we present the fundamental moth survey in medial section of the Central Mountain Range in Taiwan, Hohuanshan area, to understand (1) the influences of altitude and vegetational changes between cold and warm needle-leaf forests towards moth fauna and (2) detailed occurrence time, family composition of high altitude moths in Taiwan. Two sites, Hsiaofengko (3002m) and Chin-ma tunnel (2405m) are selected for conducting fundamental moth surveys between April, 2006 to November 2009 and Feburary 2008 to December 2009, respectively. Totally 15 families, 245 species and 4179 individuals are collected and identified. The difference in species numbers (141 and 170 species respectively) and low coefficient of similarity under Sorensen index (38.6%) reveal different moth composition, hitherto hostplant records of sampled species or cogeneric taxa in neighboring countries are adopted to infer the restriction effects of moth distribution. Moth sampling conducted more than one year uncovers different high peak of occurrence time. Moth numbers of higher site increase gradually from February and then decrease from September, however, that of lower site shows an additional peak from March to May, causing mainly in the occurrence of univoltine, spring flyer Geometridae and Noctuidae. Our works provide first comparison of moth fauna composition of subtropical high-altitude region in Taiwan.

Keywords:*timberline, moths, subtropics, Taiwan*

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PS3M268

Ecology

P3

Effect of genital spines on male fitness in the West Indian sweet potato weevil

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In many animal species, including insects, the morphology of the male genitalia is diverse. One of the most enigmatic observations in evolutionary biology is the evolution of morphological traits of male to physically injure female. It is unclear why males inflict the wound, but it has been suggested that males evolve harmful traits to induce favorable responses for males, such as delayed remating or increased rates of oviposition. The male West Indian sweetpotato weevil, Euscepes postfasciatus (Fairmaire) (Coleoptera: Curculionidae), possesses spines on the endophalus of the intromittent organ, and this spiny area is able to divide into two types. One is a band-shaped area with many short spines, and the other is an island-shaped area with several long spines. Both the standardized shape of the spines or the number of spine are significantly different between the two areas. These traits of male genital spines would affect the male reproductive success through the response of the partner. To explain the role of harmful male genital traits, we investigate the relation between the morphological character of male genitalia and fitness in E. postfasciatus. Furthermore, we investigate the narrow-sense of heritability and the phenotypic and genetic correlations for some traits. We will discuss the adaptive significance of the harmful genitalia in this weevil.

Keywords:*genitalia, shape, morphometrics,*

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Ecology

P3

Boldness, mate choice and bacterial resistance in the field cricket *Gryllus integer*

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Recent theory predicts that personality traits contributing to resource intake rates could reflect individual's condition-dependent capacity to resist pathogens. Since females often prefer mates with strong immune defence, females could potentially gain fitness benefits by using male's behavioural type (BT) as one mate choice criteria. We studied if females in the field cricket Gryllus integer would base their mate choice on male general boldness and if the boldness would predict survival under experimental challenge to opportunist bacterial pathogen Serratia marcescens. In addition, we tested if different females would prefer the same males. In general, females showed tendency to prefer bold males. Our results suggest that females may show preferences for certain BTs, but that male boldness may not reflect his capacity to resist bacterial pathogens.

Keywords:*consistent behaviour, parasite, pathogen, sexual selection*

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PS3M270

Ecology

P3

Variation in supercooling capacity of *Tribolium confusum* (Coleoptera: Noctuidae), *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Trogoderma granarium* (Coleoptera: Dermestidae)

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Physical control methods, such as low temperatures, have been extensively used to control stored product insects. Herewith, we examined the cold tolerance of the confused flour beetle, Tribolium confusum Jacquelin du Val (Coleoptera: Tenebrionidae), the sawtoothed grain beetle, Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) and the Khapra beetle, Trogoderma granarium Everts (Coleoptera: Dermestidae), using measures of supercooling point relative to developmental stage. Generally, all species presented an enhanced capacity of supercooling, as supercooling points ranged from -16.8 to -22.6℃. Developmental stage appeared to affect the superooling ability of all species significantly. More specifically, adults of O. surinamensis and T. granarium displayed a significantly higher ability to supercool in relation to its respective larvae. Only in the case of T. confusum, adults showed a decreased supercooling capacity (-16.8℃) in relation to larvae (-19.1℃). Moreover, adults of T. confusum were less capable to supercool in relation to those of O. surinamensis and T. granarium, while no significant differences were observed between larvae of the different species. Furthermore, we measured the latent heat which was released right after individuals were supercooled. In all species larvae released a higher amount of latent heat compared to adults. The highest amount of latent heat was observed for larvae of T. granarium (4.7℃) whereas the lowest for adults of T. confusum and O. surinamensis (1.7℃). Overall, no significant correlation between supercooling point and latent heat was observed among all the treatments. Our results are discussed in terms of using low temperatures to control these particular stored product insects.

Keywords:*supercooling point, Tribolium confusum, Oryzaephilus surinamensis, Trogoderma granarium, latent heat*

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Ecology

P3

Characterization of an atypical tree-aphid interaction model: *Phloeomyzus passerinii* (Signoret) (Hemiptera: Aphididae) on the stem of different poplar genotypes

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The woolly poplar aphid, Phloeomyzus passerinii (Sign.), lives on poplar trunks and causes cortical necroses leading to the death of trees where infestation is heavy. While this aphid is a major pest in poplar plantations in Southern Europe and the Near East, interactions between P. passerinii and poplar are poorly known. We have investigated (1) the feeding behavior of P. passerinii and (2) the impact of poplar resistance factors on colonization process. Studies were conducted on three Populus x canadensis Moench genotypes: 'I214' (susceptible), 'Brenta' and 'I45/51' (both resistant). Using histology and electrical penetration graphs (EPG) with aphids feeding on 'I214', we showed that P. passerinii fed upon the cortical parenchyma cells with an exceptional plant penetration strategy. The atypical EPG signal of P. passerinii was separated into two main phases: an intra-and intercellular pathway phase and a sustained intracellular phase in cortical parenchyma cells. The type and location of poplar resistance factors were defined by combining analyses of P. passerinii's performance and probing behavior (EPG) on the three P. x canadensis genotypes. P. passerinii encountered no resistance factors before its stylet reached the cortical parenchyma layer. Both resistant genotypes showed parenchyma-located antibiotic properties but characteristics were distinct according to the genotype. The genotype 'Brenta' showed additional parenchyma-located antixenosis.

Keywords:*resistance, histology, bark, EPG*

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Ecology

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Effect of temperature on development and growth of *Propylea japonica* and *Harmonia axyridis* reared on *Megoura japonica*

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Megoura japonica feeds on a variety of fabaceous plants in the genus Vicia and Lathyrus. Propylea japonica and Harmonia axyridis are indigenous to China, and also very important aphidophagous coccinellids. Since M. japonica was so easy for laboratory mass-rearing in broad bean plants as well as high reproduction, it may be useful for laboratory mass-rearing of P. japonica and H. axyridis. Preimaginal development, survival, sex ratio, size and weight upon reaching the adult stage of P. japonica and H. axyridis, which fed on M. japonica on broad bean plants, were examined at three constant temperatures (20, 25 and 30℃) and 16L: 8D. Total preimaginal development ranged from 22.5 to 24.2 days at 20℃, and to 9.3 and 14.1 days at 30 ℃ in P. japonica and H. axyridis, respectively. The larval and pupal survival decreased with the increasing of temperature. Larval and pupal survival was highest at 20 ℃ reaching 89.7 and 89.7%, and 90.0 and 86.7%, lowest at 30 ℃ reaching 60.7 and 45.0%, and 72.7 and 54.6% in P. japonica and H. axyridis, respectively. Heavier and larger adults of P. japonica and H. axyridis were obtained at 20℃, while temperature did not significantly affect the adult weight and size during 25 to 30 ℃, though the ladybirds were heavier and larger at 30 ℃. The sex ratio of P. japonica and H. axyridis was 0.44, 0.55 and 0.40, 0.53, 0.50 and 0.33 at 20, 25 and 30℃, respectively.

Keywords:*Propylea japonica, Harmonia axyridis, Megoura japonica, development, biological control*

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Ecology

P3

Foraging decisions of insect parasitoids under the threats of intraguild predation

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Aphids are attacked by a large guild of natural enemies. Intraguild (IG) interactions are widespread in aphid natural enemy communities. Immature stages of aphid parasitoids often become IG prey in interactions between predators and parasitoids operating in the same guild, and so adult parasitoids would benefit by developing avoidance responses to cues from IG predator species living in the same habitats in order to reduce predation risks to their offspring. Diverse tactics of IG predator avoidance by aphid parasitoids are shown in different two systems related to pea aphid and cowpea aphid. These tactics are 1) IG predator avoidance using chemical cue left by the ladybird Coccinella septempunctata by aphid parasitoids, Aphidius and Praon species, 2) avoiding IG predation by allocating much more searching time of aphid parasitoid, Lysiphlebus fabarum, in patches with foot stamp of ants that protect aphid colonies against IG predators, and 3) host manipulation by immature parasitoids making parasitized aphids move to ground surface from plants just before aphid mummification.

Keywords:*parasitoid, intraguild predation, predator avoidance*

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Ecology

P3

Mite predator-prey relationships in citrus could be disentangled by use of Multiplex PCR

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Multiplex PCR has been proved to be a useful molecular tool to detect and identify the citrus-feeding tetranychid mites Tetranychus urticae and Panonychus citri, and their main natural enemies, which in the Region of Valencia (Eastern Spain) are Euseius stipulatus, Phytoseiulus persimilis, Neoseiulus californicus, Neoseiulus barkeri, and Typhlodromus phialatus. Squash-capture and tissue-print of field-collected phytoseiid mites followed by Multiplex PCR has revealed to be a valid alternative to classic taxonomy for mite identification and has allowed a better knowledge of mite trophic interactions occurring in citrus orchards. Furthermore it has allowed to establish prey preferences under real field conditions. Under these conditions, the generalist pollen-feeder E. stipulatus behaves as an omnivorous species that does not seem to prey preferentially on T. urticae even when this species is highly abundant, whereas the specialist P. persimilis clearly prefers T. urticae even when this prey is scarce.

Keywords:*Tetranychidae, Phytoseiidae, Tetranychus urticae, Panonychus citri, mite trophic interactions, biological control, taxonomy, prey food preferences*

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Ecology

P3

Visual and chemical cues attracting rednecked cane borer, *Agrilus ruficollis*, to primocanes

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Blackberry production in the eastern US faces an insect pest that threatens nearly all the 7,100 tons of fruit produced annually from 2,500 acres of blackberries in the eastern North America. The rednecked cane borer has been shown to affect nearly 72% of blackberry canes and predisposes galled canes to winter injury. This pest only oviposits onto blackberry primocanes. Experiments were conducted to determine the visual and chemical cues attracting the pest to primocanes. Visual cues consisted of testing cane and leaf color mimicked traps. Traps consisting of primocane (leaf and cane), floricane (leaf and cane), yellow, purple, and control colors were placed in two locations to test for attractiveness of the trap. Chemical cues were tested by collecting plant volatiles from primocanes and floricanes, analyzing the collected volatiles on GC/MS and testing response by GC/EAD. Results from the trapping experiment showed a significantly higher number of borers trapped on the primocane and floricane colored cylindrical cane-mimic traps when compared to the control ($P < 0.05$), but not for rectangular leaf-mimic traps. Results from the chemical cue will show there is a volatile profile from primocanes that elicits an antenna response that is not observed from floricanes. Further research into developing the visual and chemical cues for this pest is necessary to develop effective monitoring programs and/or trapping systems to reduce pesticide applications.

Keywords:*rednecked cane borer, blackberry*

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Ecology

P3

Oviposition strategy of the winter cherry bug in response to egg parasitism risk

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In organisms providing no parental care, survival and development of offspring strongly depend on both timing of oviposition and choice of oviposition site by mothers. Under natural conditions, the most appropriate oviposition timing and site can change in a situation-dependent manner because biotic and abiotic factors affecting survival and development of offspring often fluctuate spatiotemporally. The winter cherry bug, Acanthocoris sordidus (Thunberg) (Heteroptera: Coreidae), has a unique oviposition strategy. Females of A. sordidus deposit their eggs not only on host plants but also off host plants. On Amami Island, Japan (28°24'N, 129°35'E), most egg clusters of A. sordidus are observed on fallen leaves around the base of host plants. Our previous study showed that the parasitism rate of eggs by Gryon philippinense (Ashmead) (Hymenoptera: Scelionidae), which is a predominant parasitoid, is lower on fallen leaves than on host plants. On the other hand, oviposition off host plants increases nymphal mortality by imposing host searching costs on nymphs. We investigated whether A. sordidus females of Amami Island population change oviposition behavior in response to egg parasitism risk by G. philippinense. Our study revealed that A. sordidus's oviposition rate was lower in the presence of parasitoids than in the absence. In addition, females which had been enclosed with parasitoids 24h just before a trial showed stronger preference for fallen leaves as oviposition substrate than females which had not. We concluded that A. sordidus females can increase their fitness by spatial-temporal avoidance of G. philippinense.

Keywords:*host-parasitoid interaction, plastic oviposition behaviour, parasitism risk, memory, Acanthocoris sordidus, Gryon philippinense*

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Ecology

P3

Differential responses to predator cues between two mosquito species breeding in different habitats

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Natural selection favours females who can correctly assess the predation risk and hence avoid high-risk oviposition sites and reduce the mortality rate of their offspring. Despite the potential significance of such behaviour, relatively few studies have assessed the relationship between oviposition behaviour and predation risk. The present study aimed to determine the sublethal effects of predators on oviposition site selection by gravid females, the foraging activity of larvae, and the life history traits of 2 mosquito species that breed in different habitats, Aedes albopictus (container breeder) and Culex tritaeniorhynchus (wetland breeder). Female C. tritaeniorhynchus avoided laying eggs at oviposition sites in the presence of a predator cue. In contrast, female A. albopictus laid eggs in both the absence and presence of the predator cue. To examine the effects of predator cues on larval behaviour, experiments were conducted in the absence and presence of a predator cue. Although larval activity was lower in the presence of the predator cue than that in its absence in both species, C. tritaeniorhynchus responded to the predator cue more strongly than A. albopictus. Female A. albopictus that had been reared with caged predators exhibited an extended larval development period, whereas the adult C. tritaeniorhynchus reared in the presence of predators were smaller than those reared in their absence. This finding might explain why C. tritaeniorhynchus avoid laying eggs in predator-conditioned water; i.e., to increase the fitness of their offspring, but A. albopictus either cannot detect predator cues or are not sensitive

Keywords:*predator kairomone*

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Ecology

P3

The distribution and diversity of butterfly (Lepidoptera) assemblages in King Saba Dalindyebo (KSD) Local Municipality, Eastern Cape, South Africa

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Butterflies (Lepidoptera) have attracted more attention as indicators of terrestrial ecosystems than other invertebrates. The study determined butterfly species turnover at a-priori-selected habitat patches in Nduli Nature Reserve and its surrounding (non-protected) landscape within the KSD Local Municipality and their response to measured environmental variables. Using the line transect survey methods, 516 butterfly individuals belonging to 22 species were recorded from 16 sampling units. Species dominance curves showed more butterfly species evenness at sites outside the reserve area than inside. Also, habitat patches outside the Nduli Nature Reserve were richer in butterfly species and overall abundance than inside the reserve. Hierarchical clustering using Bray-Curtis similarity matrices grouped sampling units according to butterfly indicator species sampled. Furthermore, Canonical Correspondence analysis (CCA) results indicated that certain site variables such as percentage herb cover, area of patch size, average grass height, grazing intensity, and average flower density accounted for species distribution patterns across sites. The conservation implications of the study suggests that patch level management of habitats with sufficient flowering herbs and other structural vegetation within and outside the reserve area can encourage both rare and common butterfly species.

Keywords:*Butterflies, species, assemblages, richness, abundance, terrestrial, ecosystem*

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Ecology
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Collective decision making during house hunting in the small-colony ant *Myrmecina nipponica*

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Social insects organise group actions, such as colony relocation, through collective decision making processes. The choice of a place to live is a critical collective decision with profound fitness implications for all colony members. This process has been intensively studied in honeybees and Temnothorax ants, but data on other species are lacking. In this study, I examined the process of collective decision making during colony relocation in the ant Myrmecina nipponica, a small colony ant. Relocation was characterised by a multi-step process; approximately half the colony acts as scouts, searching the available area independently. Gradually a pheromone trail is established to a preferred site, which leads to an accumulation of ants at the new site. This in turn produces a quorum response that is highly dependent on colony size, and a switch to brood transport occurs. The final phase of brood transport and movement of remaining individuals is relatively rapid. There was no tandem-running or carrying behaviour observed, and all individuals made their own way to the new site. Compared to other taxa examined, relocation in M. nipponica is characterised by a high level of individual assessment and apparently limited information exchange, though pheromone trails are clearly important in facilitating navigation to the new site and may be one of several positive feedback mechanisms.

Keywords:*swarm intelligence, fission, quorum, positive feedback*

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Ecology
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Host plant preference for nesting by the Japanese foliage spider, *Cheiracanthium japonicum* (Araneae: Miturgidae)

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A Japanese foliage spider, Cheiracanthium japonicum, builds nests with plant leaves. The nests are classified into seven types in relation to the seasons and purposes of nesting. We investigated whether the spider selects a plant species according to the nest type and how the physical traits of leaves influence the spider's plant selection. The difference between the composition of the host plant species used for nesting and that of the seasonal vegetation cover was confirmed. This suggests that the spider chooses the host plant species regardless of its abundance in their habitat. Early juveniles use small living or large dead leaves of various plant species to build molting nests. Females prefer long and large leaves found in Miscanthus sinensis and Phragmites japonica over other plant leaves to build breeding nests.

Keywords:*Cheiracanthium japonicum, Japanese foliage spider, host preference, silken nest, host selection*

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Ecology

P3

Effects of plants growth promoting rhizobacteria (PGPR) and fertilization on three *Brassicaceae* species and growth performance of *Spodoptera litura*

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The objective of this study was to assess the effect of plant growth promoting rhizobacteria (PGPR) and fertilizer on three Brassicaceae species [cabbage (Brassica oleracea L. var. capitata L.), Chinese cabbage (Brassica pekinensis L.) and radish (Raphanus sativus L.)] and growth performance of cutworm Spodoptera litura. Three plants were growth from seeding in the greenhouse. To evaluate the effects of PGPR (application method) and fertilization on plant's performance, four treatments were included in this study: (1) PGPR (drench and seed coating) with fertilizer, (2) only PGPR (drench and seed coating), (3) only fertilizer, and (4) control. The results revealed that plant biomass (root and shoot weight) were higher in both PGPR-seed coating and PGPR-seed coating with fertilizer treatments. To evaluate the effect of foliage on growth performance of Spodoptera litura on the three plants, four treatments were conducted: (1) PGPR-seed coating with fertilizer, (2) only PGPR seed coating, (3) fertilizer only, and (4) control. The results showed that those larvae fed on PGPR-seed coating with fertilizer treatment had better performance, higher growth rate and food processing efficiency. We concluded that PGPR in combination with fertilizer can increase plant's biomass and may play an important role in affecting the growth performance of Spodoptera litura.

Keywords:*plant growth promoting rhizobacteria (PGPR), fertilizer, growth performance, Spodoptera litura*

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Ecology
P3

Consequence of bio-diversity of aquatic organisms in paddy fields: Influence of two aquatic organisms on rice plant growth

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In recent years organic farming is well-known as the environmental friendly agriculture. Although many researches done in paddy fields have showed the difference in arthropod abundance between conventional and organic fields, there are few studies to reveal whether the abundance in aquatic organisms was influenced by the farming system and the effects of aquatic organisms on terrestrial arthropod abundance through rice plant growth. We have conducted field studies in conventional and organic paddy fields to compare the abundance in the arthropods between the two fields. The abundance of mosquito larvae was higher in conventional fields, while the abundance in aquatic predators was difference in conventional and organic fields. Abundance in mosquito larvae might influence on abundance of polypagous organisms like spiders as numerical response after they emerged adults. Results revealed that abundance in spiders at conventional fields was higher than organic fields where the abundance in larval mosquitoes was low. In addition, many pond snails were observed only in organic fields, although there were a lot of tadpoles in both fields. We carried out 2x2 factorial experiments to reveal how presence of a snail and tadpoles influences on rice plant growth. Growth of rice plants at the treatments with a snail and tadpoles tended to be higher than other treatments. We would discuss the influence of aquatic organisms on abundance of terrestrial arthropods and rice plant growth.

Keywords:*cross-ecosystem, bottom-up effect, paddy field*

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PS3M283

Ecology

P3

Symbiotic gut protists are not harbored in termite queens and kings with mature reproductive organs

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In termites, eusociality involving reproductive division of labor and symbioses with cellulolytic gut microbes are two major biological features of great interest to many entomologists. However, these features have been recognized as independent phenomena, probably due to a lack of understanding of the relationship between gut symbionts and reproductive castes. In this study, to determine the relationship between symbiotic protists and reproductives of Reticulitermes speratus, we measured the number of gut protists (including Trichonympha agilis, Pyrsonympha grandis and Dinenympha exilis) and size of guts and reproductive organs of primary and secondary reproductives together with other castes in different stages of colony development. The primary reproductives with non-developed ovaries and testes had a large amount of gut protists and well-expanded hindguts in an incipient colony. On the other hand, a small amount of gut protists and reduced hindguts were observed in the primary reproductives with well-developed ovaries and testes in a mature colony (400 days after colony foundation). Secondary reproductives collected from field colonies rarely had gut protists while workers and soldiers had a large amount of gut protists. Therefore, gut symbionts are harbored in non-active reproductive castes (alates and primary reproductives in incipient colonies) and sterile castes (workers and soldiers) rather than active reproductive castes (primary and secondary reproductives in mature colonies). The reduction of hindgut size related to the disappearance of gut microbes ensures space for reproductive maturation, suggesting that the maintenance of gut symbionts works as a suppressive factor of reproduction during the course of termite evolution.

Keywords:*termite, symbiosis, eusociality*

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Ecology

P3

Structure and function of the large pronotal horn of the sand-living anthicid beetle *Mecynotarsus tenuipes*

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Large insect horns function as antipredator armaments, digging implements, and intraspecific combat weapons. The sand-living anthicid beetle Mecynotarsus tenuipes possesses a large horn on the pronotum. Allometric relationships between body size and horn size did not show a slope of more than 1 or sexual dimorphism, suggesting another function of the horn function other than sexual selection via combat. Behavioral observation of individuals using a micro-video camera indicated that the horn is used to dig and move forward in loose sand. Only the horned M. tenuipes could dig into the sand, in contrast to the hornless anthicid beetles Stricticollis valgipes and Clavicollis fugiens, which could not dig. When moving in sand, M. tenuipes joins its pronotal horn and head to form a conical shape, with which it pierces into the sand. Then, it opens its horn and head outward to create a space in the sand for forward motion. Although it can dig deep into sand by repeating these behaviors sequentially, digging speed tends to slow with depth, probably because the weight of the substrate increases.

Keywords:*allometry, Anthicidae, digging behavior, digging cost, functional morphology*

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Ecology

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Effect of global warming on occurrence of the maize orange leafhopper, *Cicadulina bipunctata*

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The maize orange leafhopper, Cicadulina bipunctata is a serious pest of forage maize, causing maize wallaby ear symptom. This insect is originally distributed in tropical and sub-tropical regions in Africa, India and Southeast Asian countries; however its distribution has expanded to southern part of Japan since 1990s probably because of global warming. We examined effects of climatic factors (temperature, precipitation and sunlight) on the occurrence of C. bipunctata. Seasonal occurrence of adult C. bipunctata in a census field from July to August, when forage maize was most susceptible to the pest, could be described by a simple exponential function with two parameter: estimated density of C. bipunctata on 1 July (N0) and intrinsic rate of natural increase (r) for each year. Forward stepwise multiple regression analysis using seasonal occurrence data from 2004 to 2009 detected positive contributions of average temperatures in the previous December and February and a negative contribution of total precipitation during the previous winter to N0. The analysis also indicated that average temperature in July of the current year and N0 contributed positively and negatively to r, respectively. These results indicated that high temperatures and little precipitation during winter and high temperatures in early summer induced high occurrence of C. bipunctata in summer. A prediction model based on these factors supported the actual seasonal occurrence in 2010, suggesting that this prediction model is applicable to C. bipunctata forecasting.

Keywords:*Cicadellidae, Forage crop, Global warming, Pest forecasting, Regression analysis*

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Ecology

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Males kill their mating partner in a mass-reared population of the sweetpotato weevil *Cylas formicarius*

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Variation in mating opportunity often involves evolutionary change due to male manipulation during mating to assure paternity. If the female mating system changes to polyandry, the males would change the mating traits and/or mating behaviors. In an eradication program for using a sterile insect technique of the sweetpotato weevil, Cylas formicarius, in Okinawa, Japan, the weevils have been mass-reared artificially for over 90 generations. In this weevil, females do not secrete the sex pheromone after her first mating and thus the females will be monandry in the field condition. However, under the high-density such as the mass-rearing conditions, females could not avoid mating by many males after her first mating, and males can adopt several manipulations to avoid the risk of sperm competition. To avoid the remating for females, the mating will be the cost for females caused by the male inhibitions of female remating and the manipulative traits of mass-reared males will have been evolved; therefore we examined the effect of the mating with mass-reared males on female fitness. In the results, we found that some of mass-reared males killed their mating partner. Furthermore, we also found that the ejaculated semen of some mass-reared males reduced the female longevity by injecting male genital extracts into virgin female abdomens. We discuss adaptive significant of the results and paternity assurance of these traits with the males reduced the female longevity.

Keywords:*Seminal fluid, sexual conflict, longevity of females, mating behavior, multiple mating.*

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Ecology

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SEASONAL MONITORING OF THE INCIDENCE AND FLIGHT ACTIVITY OF THE GROUNDNUT LEAF MINER (*APPROAEREMA* SPP.) IN 5 SITES IN SOUTH AFRICA

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Groundnut leaf miner (GLM) (Approarema spp.) is a relatively new pest on the African continent. In South Africa, it was first noted in 2000, and has since spread over the entire groundnut production areas of the country. A study was conducted in which the infestation of GLM in groundnut and the GLM flight activity were monitored from November 2010 to February 2012 at Bhekabantu, Manguzi, Nelspruit, Brits and Vaalharts. The flight activity was monitored using pheromone traps and the infestation on groundnut was monitored by scouting in a two-planting date experiment in two seasons (November 2010 and January 2011 in first season and in November 2011 and January 2012 in the second season). Additionally, a plant-host preference experiment was conducted in the 2011-2012 season, in which infestation of GLM was monitored on, groundnut, soybean, lucerne, pigeon pea and lablab, at five sites. Vaalharts had the most male GLM captured (42%) followed by Nelspruit with 31%, Manguzi 12%, Bhekabantu 10% and Brits 5%. The highest male peak capture in all the sites was between January and April. Males were caught throughout the year but the numbers were low in winter. Pest infestation on groundnut was late, occurring only 5 or more weeks after crop emergence. Soybean had the highest infestation rate followed by groundnut at all sites. Infestation on Pigeon pea was very low occurring on one to 5 plants per site. Lucerne was scarcely attacked by the pest. Infestation by GLM was not observed on lablab at all sites.

Keywords:*Groundnut leaf miner, pheromone traps, scouting, alternative hosts, planting date*

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Ecology

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Predicting the effect of climate change on community structure and function: an assessment using grassland thrips

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Predicting how an ecosystem will respond to changes in climate is important when considering how to manage an area to reduce biodiversity loss. A common approach is to collect species abundance data from various climates, fit a model and use that to predict abundances under future climate scenarios. We will extend this idea by using recent developments in multivariate statistical modelling to predict how large communities will respond to climate change. This approach will provide more information than simply fitting separate models to each species, as extra power will be given to rare species predictions and we will be able to better assess interactions between species responses. In addition, a recently developed forth corner modelling technique will be used with morphology data to determine why particular species or individuals would respond the way they do. This will also test the idea that an assessment of trait diversity within a community can be more important that the species composition. For this assessment we are assessing Thysanoptera communities associated with Themeda australis dominated grasslands. The use of this host plant also provides the opportunity to assess the importance of ploidy in terms of structuring insect-plant interactions.

Keywords:*Climate change, Thysanoptera, Fourth corner, Grassland*

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Ecology

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Adaptive significance of homing behavior in male scoliid wasps (Hymenoptera: Scoliidae)

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Homing and site fidelity are widely known for social and nesting Hymenoptera. However, such habits of non-nesting Hymenoptera, especially in males, have rarely been studied. We focused on the homing and site fidelity in males of scoliid wasps, which are non-nesting ectoparasitoids of scarabaeid grubs. We observed the males patrolling around a small specific area, and considered that they could show strong site fidelity and homing to return to their own patrol area. Accordingly, we addressed the following questions: (1) Do males have site fidelity and return to their own patrol area from an artificially displaced area? (2) Is the patrolling area a birthplace of the males and females? (3) Why does a male persist in a birthplace? (4) Do males repeatedly move between feeding and patrolling areas? Basing on a series of field experiments, we suggest that the male wasps have homing ability and strong site fidelity to their own patrol area, and propose that this homing ability has evolved in a specific condition that males need to stay in a birthplace for mate search and to move between feeding and patrolling areas. Unlike the case in the other social and nesting Hymenoptera, homing behavior in males of non-nesting Hymenoptera should have a different evolutionary background. Therefore, the finding of homing behavior in male scoliids and clarification of its adaptive significance are important to reveal the evolution of homing behavior in the Hymenoptera.

Keywords:*philopatry, learning, parasitoids, mark recapture, mating tactics*

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Ecology

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Plant host differences in the genus *Cossus* (Cossidae): insights from mechanical tests and molecular phylogeny

Luis Fredd Leonardo Vergara Montalvo[1], R. Craig Everroad[2], Guadalupe Andraca[3], Jun Kikuchi[4], Hiroshi Makihara[5]

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Larvae of the family Cossidae are mostly associated with trees. Cossus redtenbacheri (Hammerschmidt) is exceptional in this family because its larvae live in agave. Two approaches were followed to better characterize this host difference. First, a mechanical test was performed to evaluate the gallery making ability of larvae transferred to non-natural hosts of differing tissue hardness. Second, the first molecular evolutionary analysis of C. redtenbacheri was performed to assess its relationship with selected wood-feeding Cossidae species. This phylogenetic reconstruction was based on partial sequences of mitochondrial cytochrome c oxidase subunit 1. The results show that the host difference is not due to mechanical constraints. Phylogenetically, the agave-feeding phenotype is confirmed as a derived character within the genus Cossus. The possibility and type of ecological factors that may explain the host specialization of C. redtenbacheri are discussed.

Keywords:*28S, borer insects, CO1, Comadia redtenbacheri, Cossus insularis*

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Ecology

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Elucidating factors by which *Orius* spp. is attracted and arrested to Okra

Hojun Rim[1], Masayoshi Uefune[2], Rika Ozawa[3], Akio Nakano[4], Junji Takabayashi[5]

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Under field conditions, predatory bugs Orius spp. are often found at buds of Okra plants, Hibiscus esculentus, but not on leaves, even though the buds are not infested by their prey. The number of Orius spp. at buds is different in three cultivars of Okra (Marumi, Beni and Early five). We investigated how Orius spp. were attracted and arrested at Okra buds by using Orius strigicollis and two cultivars, Beni and Marumi. Bioassays using a Y-tube olfactometer revealed that adult females of O. strigicollis were attracted to volatiles from Marumi buds. Volatiles from Beni buds tended to attract adult females, though it was not statistically significant. Adult male O. strigicollis were not attracted to volatiles from Okra buds of both cultivars. In the rearing vial, when offered buds of Marumi or Beni, Orius survived longer than control (no buds in the vial). In contrast, when offered leaves of Marumi or Beni in the rearing vial, longevity of O. strigicollis was not significantly different from that in control (no leaves in the vial). Headspace analyses of Marumi and Beni buds showed that linalool, β-ocimene, and 3-Hexen-1-ol acetate were the major compounds. Bioassays using these compounds to clarify the active principals that attract and/or arrest O. strigicollis at buds is now under way.

Keywords:*Orius, Okra, volatile*

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Ecology

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Historical fires on grasslands in central Japan and its causation to distributions of grassland species of bumblebees and endangered butterflies

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The warm and humid climate causes ecological succession of most vegetation to forest in Japan. However, this view of the Japanese vegetation is on the assumption that human disturbance is absent. In fact, grassland species of plants and insects have survived in semi-natural grasslands from the time of the glacial periods until today. Tremendous reduction of semi-natural grasslands during the 20th century in Japan caused crucial threat to the grassland species. Many of these species are considered to be relics since glacial periods that had expanded from the Asian continent probably through the Korean Peninsula. To clarify the significance of the inferred human influence on the maintenance of habitats for these species, the history of vegetation, archaeology and Japanese history are reviewed and statistical analyses were conducted using data from recent and present insect distributions in Nagano Prefecture, central mountainous area of Japan. Existence of a black soil, called 'kuroboku', indicates the locations of the past semi-natural grasslands caused by fire since the Jomon era (the early Holocene). Many of the ruins of the Jomon era are assembled in the areas of black soil and pasture fields since the ancient age had often overlapped with those areas. Statistical analyses using generalized linear model (GLM) exhibited that distribution of endangered grassland species of bumblebees (Bombus spp.) and endangered grassland butterflies including Shijimiaeoides divinus barine coincide with the areas of the black soil. It suggests that the usage of fires by humans had historically provided continual habitats for the grassland species.

Keywords:*black soil, human disturbance, kuroboku, semi-natural grassland, Shijimiaeoides divinus barine*

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Ecology

P3

The mating system using volatile and substrate-born sex pheromones in a polyphagous solitary parasitoid wasp, Itoplectis naranyae

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Although volatile or substrate-born sex pheromones are known in some parasitoid wasps, the detail process of the mate search by parasitoid wasps has rarely been examined. The parasitoid wasp Itoplectis naranyae is distributed in Japan and the other East Asia and found in rice paddies and grass fields covered mainly by Poaceae plants. I. naranyae parasitizes variety kinds of lepidopteran pupae including rice pests. Because of solitary polyphagous parasitizing style, each sex is scattered in the environment and the mate search would be difficult in this parasitoid. However, the mating rate of wild females was shown 100%. The percentage suggested the existence of a mating system to ensure successful sexual encounter. In the previous study, we have demonstrated the existence of volatile sex pheromones secreted by the parasitoid, but the attractiveness was not enough in the field. Thus we suspected that volatile sex pheromones were not the sole cue used in the mate search and conducted a series of laboratory experiments to examine other cues involved. We found that male I. naranyae showed longer antennal sweep duration to glass walked by virgin than by mated females. Additionally, males presented more wing vibration frequency on the glass walked by young than old females. The results indicated that I. naranyae used substrate-born sex pheromones as another cue for mate search. Thus, I. naranyae uses two different types of sex pheromones to overcome difficulty in mate location. The combination of two different cues would ensure successful sexual encounter.

Keywords:*mating behavior, sex pheromone trap, bioindicator, Ichneumonidae*

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Ecology

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Effect of bush burning on the egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) of the endangered butterfly, Shijimiaeoides divinus barine (Leech) (Lepidoptera: Lycaenidae)

Keiko Koda[1], Hiroshi Nakamura[2]

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The large shijimi blue, Shijimiaeoides divinus (Fixsen), is a grassland lycaenid butterfly, which is distributed in Japan, the Korean Peninsula, Northeastern China and the Southern Amur region. This butterfly was designated an Endangered Species by the Ministry of the Environment of Japan. Only small populations of the subspecies S. divinus barine remain in limited areas of Nagano Prefecture in Japan. The volunteer organization has been in active to recover the natural population of this butterfly by the method of releasing pupae. However, the natural population was not recovered. We clarified that the egg parasitoid, Trichogramma chilonis was an important mortality factor of the egg stage of S. divinus barine. Bush burning experiment was carried out in Azumino City, Nagano Prefecture on March 29, 2009 in order to verify the effect of bush burning on the egg parasitoid. Immediately after bush burning, a cage was set up in the bush burning area and the control area respectively. The percentage of parasitism inside the cage was 2.3% in the bush burning area and 30.3% in the control area ($P<0.01$). It was given as a conclusion that the bush burning had an effect on parasitism of T. chilonis on S. divinus barine eggs. In Alps Azumino Natural Government Park in Azumino City, bush burning has become to be performed by the results of our researches every year. As a result, S.divinus barine adults were recovered in 2011 after the 15 years' separation in this area.

Keywords:*Bush burning, Azumino, Japan, Egg parasitism, Trichogramma chilonis, Shijimiaeoides divinus barine, Endangered Species*

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Ecology

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Physiological response of strawberry (Fragaria x ananassa Duch. cv. 'Yunia Smuds') to spider mite (*Tetranychus atlanticus* McGregor, 1941) feeding

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Plants were grown in a greenhouse at 20-24ºC, 65-85% RH, and a photoperiod of 16:8 (L:D) h. Each leaf was infested with 10 spider mite protonymphs. Population dynamics were counted on 2th, 9th and 16th day after the infestation. These data were used for calculation of mite-days. There were equal to 3,9±0,3, 66,2±6,9 and 86,8±20,7 for 2, 9 and 16 days, correspondingly. Non-photochemical quenching of chlorophyll fluorescence (NPQ) after 2 days was the same as in the control. On 9th and 16th days NPQ consistently increased as compared with controls. The thermoluminescence intensity (TL) in the temperature range from -40 to +30ºC for 2 and 9 days was slightly lower than in controls, and significantly decreased after 16 days. Although measurements of fluorescence parameters indicated a disruption of photosynthetic apparatus, chlorophyll content was not affected by spider mite feeding. Mite feeding caused a mechanical injury that was restricted to the spongy mesophyll with minimal damage to the uppermost palisade layer. This may explain the lack of relationship between chlorophyll content and injury. In injured leaves the intensity of the high-temperature TL (≥ 60ºC) after two days was the same as in the control, after 9 days intensity increased as compared with the control and remained the same on the 16th day. The growth of high-temperature TL may indicate a partial degradation of membrane lipids during oxidative stress. This effect may be due to reduction in leaf gas exchange parameters, especially CO2 fixation with the resultant generation of ROS.

Keywords:*spider mites, non-photochemical quenching, thermoluminescence, ROS*

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Ecology

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Changes of the land use and decline of the endangered butterfly, *Shijimiaeoides divinus barine* in Azumino, Japan

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[1]Shinshu University, Japan, [2]Shinshu University, Japan, [3]Nagano Environmental Conservation Research Institute, Japan

The large shijimi blue, Shijimiaeoides divinus barine is distributed in limited areas of Nagano Prefecture in Japan at present. It has been designated an Endangered Species by the Ministry of the Environment of Japan. A large population of this butterfly was observed till 1960. However, the population of S. divinus barine decreased with the changes of the management methods (mowing and bush burning) of inhabitant area. We clarified that the bush the burning had a good effect on survival of S. divinus barine. When did bush burning begin in Japan? The andosol which was formed by the bush burning in the Jomon period (BC10000-BC3000) is widely distributed in Nagano Prefecture. It is known that the distribution areas of S. divinus barine overlapped the andosol and the imperial pastures (about AD900). The semi-natural grassland which is suited to S. divinus barine has been maintained from the Jomon period by pasturage or bush burning in Nagano Prefecture. However, after the Edo period, the paddy field was reclaimed in Azumino, and the pastures were lost (about AD1800). Was S.divinus barine exterminated? Sophora flavescens, the host plant of this butterfly, was planted in ridges of a rice field and the bank of the irrigation canal as a herb. Mowing and bush burning were performed periodically there. Thus, the habitat of S.divinus barine has been maintained. However, the land improvement enterprise was undertaken around from 1962. Sem-natural grassland vegetations including S. flavescens were lost and S.divinus barine can live has disappeared.

Keywords:*Shijimiaeoides divinus barine Azumino, Japan, endangered butterfly, land use, decline*

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Ecology

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Effect of grazing intensity on species diversity of butterfly communities

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We conducted route census surveys on butterfly communities in the grasslands under different grazing intensities on the volcanic slopes of Mt. Aso, Japan. At the study sites, the semi-natural grassland underwent routine burning every spring. The results obtained are summarized as follows: (1) Sixty-four species assigned to five families were recorded. (2) Ten "rare species" nominated in the "red list" were recorded. (3) Grassland species, Shijimiaeoides divinus asonis and Colias erate poliographys. And forest species. Celastrina argiolus labonides, Ypthima argus and, Eurema mandarina were abundant. (4) More species were found in the non-grazing site (site NG) than in the customary grazing site (site CG), and more forest species were found in the site NG than in the site CG. (5) Shimpson's index of diversity (1-λ) was higher in the site NG than in the sites CG. (6) S. divinus asonis was the most abundant species at the site CG. (7) Shimpson's index of diversity (1-λ) decreased as the grazing intensity increased. (8) Grazing intensity didn't affect the number of rare grassland species. The number of rare forest species at NG was the highest among the four study sites. (9) The ratio of S. divinus asonis to all butterflies was about 90 % in the sites NG, LG, and CG. In conclusion, mixture of grasslands under various grazing intensity may have a benefit for the maintenance of diversity of butterfly fauna. Especially, grasslands maintained without grazing

Keywords:*Large Shijimi Blue, Grazing intensity, Butterfly communities*

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Ecology

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Effects of jasmonate and salicylate induced defense in wheat on grain aphid, *Sitobion avenae* (Hemiptera: Aphididae)

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Jasmonate and salicylate-mediated signaling pathways play significant roles in induced plant defenses. We studied biochemical responses and effects on grain aphid, Sitobion avenae (Fabricius) when exogenous applied methyl jasmonic acid (MeJA) and salicylic acid (SA) on wheat. Application of MeJA significantly increased levels of polyphenol oxidase (PPO) and proteinase inhibitor 1, 2 and 6 days after treatment. In two choice tests, adult aphids prefer untreated leaves to MeJA- or SA-treated leaves, and the weight of apterous adult aphids reared on MeJA treated seedlings were reduced significantly as compared with those on untreated seedlings. Electrical penetration graph (EPG), however, showed that the stylet activities of aphids had similar patterns on all treated plants. Total probing time and phloem phase time on MeJA- and SA-treated plants were not different from those on untreated plants. Duration of the aphid's first probe on MeJA treated plants was significantly shorter and the aphid's salivation period phase on SA treated plant was longer as compared with those on other plants. These findings advance the knowledge of the effects of JA-dependent and SA-dependent defenses in wheat on phloem-feeding insects. Our results suggest that both MeJA and SA applied to wheat seedlings negatively influenced aphid colonization processes.

Keywords:*Methyl jasmonate Salicylic acid Induced plant response EPG Wheat Sitobion avenae*

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Ecology

P3

Phenotypic and genetic differences among exotic herb *Solidago altissima*: how do they respond to reassociation with the exotic insects ?

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Introduced species often quickly change in terms of genetic variation and phenotypic traits due to adaptive evolution. However, the time scale responding to the trait change after invasion is poorly explored. The recent expansion of an invasive herbivorous insect Corythuca marmorata, which was firstly recorded in 2000 in Japan, of the exotic plant Solidago altissima provides an opportunity to test the potential of the selective impacts of this herbivore on plant defensive traits. Because the invasion process of C. marmorata differs geographically in Japan, the defensive traits of S. altissima may have locally evolved. We focused on the geographic variation of the insect species, abundance, and genetic and phenotypic differences among S. altissima populations in a wide range of Japan. The averaged damage by C. marmorata was lower in populations where C. marmorata was initially introduced than the populations in the recent expanded range. Moreover, the S. altissima populations were exclusively hexaploid, although diploid, tetraploid, and hexaploid are reported in its native range. These findings suggest that the defensive traits may have been subjected to natural selection differently in local populations in response to local biotic environments in a short time scale, providing a new insight to understanding the rapid adaptation of introduced plants in response to community composition of insect herbivores in a new habitat.

Keywords:*invasion, plant-insect interaction, Solidago altissima*

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Ecology

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A survey on oviposition behavior of *Tirchogramma brassicae*

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Several studies have proven that T. brassicae is the most dominant species in Iran with most distribution. Considering the importance of studying oviposition behavior and spent times during these behaviors and widespread use of T. brassicae in biological control programs this study was conducted to study this behavior of sexual T. brassicae. Studying of behavior was conducted under controlled condition: 25±1°C, 50±10 % RH and 16:8 L:D. Individual Trichogramma females were presented with egg patches consisting of 100 host eggs glued with water on to a 5×5 cm white cardboard square with different host quality (100 fresh and old host eggs) separately. The host eggs were separated by a distance of 5 mm. A sequence of six behavioral events was used to monitor host use by the Trichogramma females. Spent time for oviposition on each fresh egg was 70±0.45 S, while this amount on old eggs was 73±0.61 s. Observed acceptance time for each fresh egg was 15±0.29 s but this time on old eggs was 14 ± 0.44 s. Rejection time of each fresh egg was 6±0.91 s and this parameter was 7±0.77 for each old egg.

Keywords:*oviposition behavior, biological control, Trichogramma brassicae*

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Olfactory learning and memory duration of *Wolbachia* infected *Trichogramma brassicae*

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Odor learning has been studied on large number of hymenopteran species and documented for nearly 36 species of Hymenoptera. According to several studies T.brassicae is the most dominant species among Trichogramma wasps in Iran. Thelytoky has been observed base on Wolbachia infection in this species. This study was carried out to show learning ability and memory duration of wolbachia infected T.brassicae .Wasps culture was carried out in controlled condition, 25±1°C, 70±10% RH and 16:8 L: D. Twenty naive one day old wasp were introduced to odor tanks and one hundred one day old flour moth egg were exposed to these wasps for 5 hours and olfactory response of these wasps were observed in Y maze olfactometer. According to our results learning ability of this wasp was proven, maximum olfactory response was recorded till 12 hours and continued till 24 hours. Maximum memory duration period of this wasp was 24 hours after exposing to odor.

Keywords:*learning, memory duration, olfactometer, Trichogramma brassicae*

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Ecology

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Endophytic bacteria associated to sharpshooters, insect vector of *Xylella fastidiosa*: Potential of symbiotic control

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Xylella fastidiosa causes citrus variegated chlorosis (CVC) disease, resulting in significant production losses in the citrus industry. X. fastidiosa is transmitted by species of sharpshooters. We identified bacterial communities associated with the heads of surface-sterilized insect vectors of X. fastidiosa. Bacteria were isolated and analyzed by amplified ribosomal DNA restriction analysis and sequencing, revealing the presence of the well-known citrus endophytes Methylobacterium spp. and Curtobacterium spp. Paratransgenics, also called symbiotic control, is a new strategy that consists of genetic alteration in symbiotic microorganisms and the reintroduction of them into their original niche. The key to symbiotic control is finding microorganism candidates that are associated with the target plant-pathogen or pest. Based on the premise of symbiotic control, we genetically modified the citrus endophytic bacterium Methylobacterium extorquens and evaluated its capacity to colonize a model plant and its interaction with X. fastidiosa. This Methylobacterium strain was genetically transformed to express heterologous Green Fluorescent Protein (GFP) and an endoglucanase A (EglA), generating the mutant strains ARGFP and AREglA. By fluorescence microscopy, it was shown that ARGFP was able to colonize xylem vessels of host plant. Using scanning electron microscopy, it was observed that AREglA and X. fastidiosa may co-inhabit the xylem vessels of host plant and appeared to cause a decrease in biofilm formation. AREglA stimulated the production of resistance protein, catalase, in the inoculated plants. Our results provide an enhanced understanding of the M. extorquens and X. fastidiosa interaction, suggesting the application of endophytes as an agent of symbiotic control.

Keywords:*citrus variegated chlorosis (CVC), Curtobacterium sp., endophytes, Hemiptera: Cicadellidae Methyloba*

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Take-off behavior of *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) in field conditions and it's relation to temperature, illuminance and wind speed

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The aerial flight process of migrating insects roughly consists of three stages: taking-off, navigation and landing. Taking-off so played an important role as the initiation of migration. In this study we observed the take-off behavior of Cnaphalocrocis medinalis (Guenee) for summer migration both in rice field of natural state and a climate chamber. And possible relationships with certain environmental factors including ambient temperature, light intensity and wind speed were analyzed. The whole process of take-off behavior of C. medinalis occurred in 20 - 40 minutes after sunset and reached peak period at the ending of evening twilight. There were no individuals taking-off before sunset. Occurrence of the take-off behavior needed the triggering of optimum light intensity that ranged from 0.1 to 10 lx. We also show that ambient temperature and wind speed have an influence on the flight and landing behavior of C. medinalis. The ovarian development showed significant difference between emigrants and retentions, which mean that the oogenesis-flight syndrome occurred in its first long distance migration.

Keywords:*Cnaphalocrocis medinalis, take-off behavior, caged observation, temperature, light intensity, wind speed*

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Evaluation of meridic diets for rearing *Scyphophorus acupunctatus* (Coleoptera: Curculionidae)

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The weevil, Scyphophorus acupunctatus Gyllenhall, is an important pest of Agave spp. and Mexican tuberose (Polianthes tuberosa L.). Larvae feed in the tuberose bulb forming galleries and complete their development within a cocoon from which the adult emerges. Damage is estimated to be 35 to 70% of adult plants. Copulation is carried out among the bulbs, where the female lays her eggs. We evaluated 4 diets; all diets included the following: agar, vitamins, sucrose, brewer's yeast, wheat bran, cholesterol, Wesson salt mixture, sorbic acid, ascorbic acid and methyl P-hydroxybenzoate. Three diets were supplemented with different amounts of tuberose host-plant bulb and one with jicama (Pachyrhizus erosus R. T. Clausen Urb.). The tuberose bulb was used as a control. All of the diets assayed were accepted by S. acupunctatus neonatal larvae. Larvae fed with the tuberose C diet had the lowest weight while those fed with the tuberose A and jicama had the highest weights. Total survival from larva to adult was significantly high on tuberose B and tuberose C diets, while total survival for the jicama diet was considerably lower and even less than in the control. Total larval + pupal development time in the 4 meridic diet were significantly longer than on the control. Insects feed on tuberose diets had larger fecundity, fertility and oviposition rate than those on the control. These results show that one of the tuberose diets is suitable for establishment of a lab colony.

Keywords:*survival, fecundity, alternative diet*

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Seed predation process of *Megastigmus transvaalensis* (Torymidae) in *Schinus terebinthifolius* (Anacardiaceae) in Brazil

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We studied larval biology and seed predation process of Megastigmus transvaalensis on four individuals of the dioecious tree Schinus terebinthifolius, between February-April 2011/2012. For this, we experimentally manipulated fruit age and wasp oviposition time. Previously the experiments, branches containing about 100 pistilate flowers were isolated with tissue bags before receptiveness. At anthesis, stigmas were manually pollinated using a fine paintbrush impregnated with pollen. When fruits attained the ideal size for oviposition, we introduced inside the tissue bags 20-30 female wasps obtained from other S. terebinthifolius individuals. We allowed wasps to oviposit for 48h. From last oviposition day we collected 30 fruits at 2-3 days intervals up to larvae have completed their development. We manually dissected 5 oviposited seeds, recorded the larva position inside the seed and measure its body length. Oviposition occurred in fruits aged 15-20 days, when plant embryo was at its cotyledonary stage. Egg was always deposited inside the nucellus. First larval stage (0.41±0.13mm, n=20; 4-7 days) began to feed on endosperm. Seed continued its growth and second larval stage (1.01±0.37mm, n=25; 8-9 days) attacked part of the integument, endosperm and occasionally the plant embryo. The third larval stage (3.42±0.98mm, n=40; 10-15 days) consumed the mature plant embryo and occupied most of the seed cavity. We showed that M. transvaalensis development is adjusted to exploit resources derived from the plant embryological processes. Financial support: S.J.G. FAPESP#09/10237-9. R.A.S.P. FAPESP#04/10299-4 and CNPq#302769/2008-0. S.P.T. CNPq#301960/2009-7.

Keywords:*Seed predation, Larval biology, Torymidae, Anacardiaceae*

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Ecology

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Different uses of plant semiochemicals in host location strategies of two "microtype" parasitoid flies

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Some members of the Tachinidae (Diptera) deposit a large number of small eggs, termed "microtype" eggs, on the food plant of their herbivorous host. Parasitization becomes successful only when the host ingests these eggs. To increase the chance of hosts encountering the eggs, the microtype tachinids have to choose a suitable plant that harbors hosts and/or lay their eggs near the hosts. In the host location process, semiochemicals emitted by host-infested plants provide reliable cues to the tachinids. We investigated the behavioral response of two microtype tachinids, Pales pavida and Zenillia dolosa, in a wind tunnel with maize plants infested with a common host, the larvae of the noctuid moth Mythimna separata. Females of P. pavida showed a significantly higher rate of landing on M. separata-infested maize plants than on artificially damaged or intact plants. Z. dolosa landed on both the infested and artificially damaged plants with significantly higher rates than on intact maize plants. We also examined which part of the infested maize leaf induces oviposition. P. pavida deposited eggs on the margin of the leaf. In contrast, Z. dolosa intensively laid eggs around an area of host infestation or an artificially damaged spot. The eggs of P. pavida demonstrated parasitization ability over 15 days after they were deposited on plants, while the eggs of Z. dolosa did not survive more than 5 days after oviposition. Our data illustrate that each microtype tachinid employs a different host location strategy to increase the rate of parasitization success.

Keywords:*Tachinidae, parasitoid, HIPVs, tritrophic interaction, oviposition behavior*

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Ecology

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Trade-offs between pupal melanism and life-history traits in *Spodoptera exigua*

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Insect melanism has interested biologists for long time, and its study integrates diverse classical branches of biology, including ecology, development, genetics and physiology. In this study, we established a homozygous melanic strain (SEM) with black pupae spontaneously occurring within a laboratory population (SEW) of Spodoptera exigua. Morphological photograghy showed that the SEM strain is globally melanism in the pupa stage but not in the egg and larval stages. After pupation, the SEM pupae gradually turn black, while the SEW pupae gradually turn yellow-brown, indicating that melanin over synthesizes in SEM. Life-table experiments indicate that the SEM strain has faster development in all life stages, heavier pupa weight, higher mating rate and fecundity, more mating time and accordingly higher net reproductive rate and population trend index. Single pair inbreeding and reciprocal crosses reveal that there is no reproductive isolation between the SEM and SEW strains, and the reciprocal crosses rate is significantly higher than intra-cross rate of SEW strain. The color of filial generation pupae are different from their parents, and the pupae of F1 progeny from ♀SEW×♂SEM are dark-brown while the pupae of F1 progeny from ♀SEM×♂SEW are light brown. These results illustrate that Spodoptera exigua pupal melanism is an advantageous mutation. The SEM strain will be a good model organism for study on the naturally variable genotypes with the phenotypes that affect fitness in Lepidoptera.

Keywords:*Spodoptera exigua, pupal melanism mutation, biological characteristics, fitness*

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The effect of larval density on development of the coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)

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The coconut hispine beetle, Brontispa longissima is one of the most serious insect pests of the coconut palm, Cocos nucifera. This beetle is distributed in Southeast Asia, China, Japan, the Pacific Islands and Australia. Brontispa longissima shows aggregation in the field. To elucidate the effect of aggregation on larval developmental aspects, we examined the effects of larval density on various aspects of larval development and on survival rates. Recently we found that B. longissima was divided into two monophyletic clades by genetic analysis. Therefore, we also compared the results between two populations, from Ishigaki, Japan (ISH) and Papua New Guinea (PNG), which were representative of the two monophyletic clades of B. longissima. In both ISH and PNG, the larval developmental period was shorter and the survival rate higher with rearing under high density conditions than under isolated conditions. Similarly, fewer instars were required before pupation under high density conditions than under isolated conditions. Our study revealed that B. longissima developed better under high-density conditions, and the trends in the density effect were similar between two monophyletic clades.

Keywords:*coconut hispine beetle, Cocos nucifera, invasive species, Brontispa longissima*

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Ecology

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Factors associated with damage to blueberry fruits by *Drosophila suzukii*

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Drosophila suzukii is a pest of thin-skinned fruits including strawberry, blueberry and cherry. Blueberry was introduced into Japan in the 1980s, and severe economic losses attributable to D. suzukii were first reported in 2002. D. suzukii occurs in July and September around Tokyo, but is absent during August. One of the two major species, Highbush blueberry (Vaccinium corymbosum L), harvested in July, can receive serious damage from D. suzukii but the other species, Rabbiteye blueberry (Vaccinium virgatum Aiton), harvested in August, suffers little damage. Since the reasons for this difference in damage are not clear, the objective of this study was to elucidate whether temperature and blueberry variety influenced the extent of damage by D. suzukii to blueberry cultivation. Oviposition by D. suzukii females in berries of different varieties and fruit firmness was examined in the laboratory. More eggs tended to be laid in berries of cultivars having soft fruits than having hard fruits. Larval development and oviposition of D. suzukii at different temperatures were examined. Above 31 °C, larval development was prolonged and adult eclosion was inhibited. When adults mated at 25°C, 28 °C and 31°C for 4 days, the numbers of oviposited eggs were lower than those at 25 °C, and the hatching rate at 31°C was lower than those at 25 °C and 28°C. Thus, Rabbiteye blueberries appear to escape serious damage by D. suzukii because the harvest season coincides with peak temperatures in August, when larval development and adult reproductive activity are likely to be suppressed.

Keywords:*Drosophila suzukii, Highbush blueberry (Vaccinium corymbosum), Rabbiteye blueberry (Vaccinium virgatum), Japan*

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Ecology

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Aster Yellows phytoplasma strain Witches Broom alters plant development to manipulate plant-insect interactions

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Phytoplasma are pathogens that cause disease in a wide range of agricultural plants. These bacteria have a unique life cycle that involves the colonization of eukaryotic hosts belonging to two kingdoms, namely plants (Plantae) and insects (Animalia). Phytoplasma are transmitted to plants by phloem-feeding insects, including planthoppers, leafhoppers, and psyllids. Naive (uninfected) insects acquire the bacteria whilst feeding from infected plants, and remain infected for life. Accordingly, phytoplasma fitness is strongly influenced by the interactions that occur between its plant and insect hosts. Aster Yellows phytoplasma strain Witches Broom (AY-WB) can infect a broad range of plants, including Arabidopsis, and is transmitted by the aster leafhopper Macrosteles quadrilineatus. Infected plants exhibit symptoms that reflect alterations in development, including the increased production of stems ('witches broom' phenotype) and growth of green, leaf-like flowers. AY-WB-infected Arabidopsis plants also become better hosts to support the reproduction of M. quadrilineatus: leafhoppers produce approximately 60% more progeny on AY-WB-infected plants compared to healthy controls. Furthermore, the specialist corn leafhopper Dalbulus maidis survives longer and produces eggs when caged on AY-WB-infected Arabidopsis, whereas D. maidis will not oviposit on healthy plants. Our central hypothesis is that AY-WB secretes proteinaceous effectors that target host processes relevant to defence and development. We have characterized two effectors produced by AY-WB, SAP11 and SAP54, which elicit phenotypes when expressed in Arabidopsis that resemble symptoms of AY-WB infection. We discuss herein how these effectors may hijack plant development to increase plant-insect interactions to the benefit of the phytoplasma.

Keywords:*phytoplasma, Arabidopsis, leafhopper, effector*

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Ecology

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Differential pesticide susceptibility explains replacement of *Neoseiulus womersleyi* by *N. californicus* in Japanese pear orchards

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Neoseiulus californicus and N. womersleyi are important natural enemies of spider mites. In 1963, N. californicus was first collected in Japan, but no further collections were made until the 1980s. Since the 1990s, the indigenous N. californicus strain is abundant and found on a variety of deciduous trees and weeds, and it has replaced N. womersleyi in many conventional Japanese pear orchards in central and southeastern Japan. This increase of N. californicus in Japan precedes the use of a commercial strain of N. californicus (Spical®), because this strain has not been used in Japanese greenhouses and fields before 2003. In this study, we tested the hypothesis that species replacement is due to differences between N. californicus and N. womersleyi in susceptibility to agricultural chemicals used before and after 1990: N. californicus is highly susceptible to chemicals used before 1990 but less susceptible to those used after 1990, and vise versa for N. womersleyi. We compared the susceptibility of N. californicus and N. womersleyi to 22 chemicals (11 insecticides, six acaricides and five fungicides) used before and after 1990. Susceptibility of N. californicus to chemicals used before 1990 was high. In contrast, N. californicus showed lower susceptibility to some chemicals used after 1990, especially to neonicotinoid pesticides, which had significantly lower effects when compared with N. womersleyi. These results suggest that difference in susceptibility to neonicotinoid pesticides is one of the possible factors influencing species replacement from N. womersleyi to N. californicus in Japan.

Keywords:*Neoseiulus californicus, Neoseiulus womersleyi, species replacement, pesticide susceptibility*

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Stage-specific defensive strategies of three mantid species, *Tenodera aridifolia, Hierodula patellifera*, and *Statilia maculata*, against a natural enemy

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Three mantid species, Tenodera aridifolia (Stoll), Hierodura patellifera (Serville), and Statilia maculata Thunberg, of varying body sizes and habitats, exhibited defensive reactions when faced with a natural enemy, the Japanese skink [Takydromus tachydromoides (Schlegel)]. Defensive reactions were analyzed as primary or secondary defenses for all developmental stages. Of the primary defenses, the cryptic reaction was the main defensive reaction: mantids lowered the prothorax and simultaneously stretched the prothoracic legs, the antennae were arranged anteriorly, and the abdomen was sometimes extended posteriorly. The frequency of the subcryptic reaction as a primary defense tended to increase with more advanced developmental stage. The primary defensive strategy switched at a specific developmental stage and also depended on the distance between a prey mantid and the natural enemy. In some instars, T. aridifolia and H. patellifera displayed a cryptic reaction when the mantid-foe distance was long, whereas they displayed a subcryptic reaction when the mantid-foe distance was short. However, S maculata did not switch defense strategies based on the mantid?foe distance. The secondary defenses of young instar nymphs of T. aridifolia and H. patellifera were primarily defensive reactions, whereas the frequency of aggressive reactions tended to increase with developmental stage. S. maculata did not display aggressive reactions during any stage. I concluded that the primary and secondary defenses used by mantids depend on the developmental stage and mantid?foe distance. Moreover, three mantid species that vary in both body size and habitat showed different defensive reactions when faced with a natural enemy.

Keywords:*mantid, crypsis, primary defense, secondary defense*

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Ecology

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Phytoseiid mite species composition in Japanese peach orchards estimated using quantitative sequencing

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The phytoseiid mites are regarded as potential biological control agents for spider mites. Information related to phytoseiid mite species composition is important for spider mite control. However, phytoseiid mite species identification based on morphological characteristics alone is sometimes difficult, because inter-specific differences are often subtle and the collected individuals can lack distinguishing morphological features. In this study, we established the method to estimate phytoseiid mite species composition using quantitative sequencing. Ribosomal gene fragments were amplified from genomic DNA of five phytoseiid mite species inhabiting peach orchards in Okayama Prefecture using PCR. Based on the nucleotide sequences of the amplified products, species-specific polymorphic sites were identified. Newly amplified products from recombinant plasmids were mixed in various ratios to yield standard DNA template mixtures. After direct sequencing, the signal ratios between two nucleotides at each species-specific polymorphic site were calculated and shown against the corresponding expected ratios. Quadratic regression equations were used to estimate the phytoseiid mite species composition. Results reveal that the phytoseiid mite species composition is seasonally changed and varied among study sites.

Keywords:*Peach orchard. Phytoseiid mite. Quantitative Sequencing. Species composition. spider mite*

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Ecology

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Sexual behavior, chemical communication, and reproductive isolation in *Proeulia auraria* Baker (Lepidoptera: Tortricidae)

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During 2011 spring, about 1,000 Proeulia auraria larvae were collected in a vineyard and reared in the laboratory. Observations of adult females allowed determining that they perform calling behavior during the scotophase between 2 and 8 a.m., mostly after 5 a.m. The calling behavior is characterized by activation of the resting female, followed by elevation of the wings and partial exposure of the putative pheromone gland at the terminalia. Tested females performed this sequence the first three nights after emergence. Pheromone glands were excised and extracted in hexane, as well as the rest of the abdomens, at calling times. By means of gas chromatography-mass spectrometry, E11-14Ac and 14Ac were identified from glands but not from the rest of the abdomen samples. Two field trials conducted in a vineyard severely infested with P. auraria showed that males were strongly attracted to traps baited with E11-14Ac, in doses between 0.1 and 10 mg/septa*trap. However, when E11-14Ac was mixed with E11-14OH in identical concentrations (as used in commercial lures in Chile), male captures were significantly reduced. When the alcohol was used alone, captures did not differ from control traps. It is hypothesized that E11-14OH acts as an inhibitor for P. auraria males, and might be used by other synchronic and sympatric native tortricids that share similar niches (host plants), to avoid interspecific matings. We acknowledge the support to the grant FONDECYT 1110365 from the Chilean Commission for Research in Science and Technology.

Keywords:*Chermical ecology, insect behavior*

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Flexible food-searching in the parasitic wasp, *Cotesia vestalis*

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Female parasitic wasps should not only forage for hosts to increase current reproduction, but should also forage for food to increase future reproduction through their own survival. Females of the endoparasitic wasp, Cotesia vestalis, attack larvae of the diamondback moth (Plutella xylostella) to lay eggs, but they don't exploit the hosts as a food source by so-called 'host-feeding'. Under laboratory conditions, we demonstrated that female wasps could utilize non-host food sources, such as floral nectars and honeydew of a homopterous insect. Female wasps lived significantly longer on flowers of Brassica rapa than on water alone, and they also survived significantly longer when provided with plants infested with non-host green peach aphids (Myzus persicae) than when provided with uninfested plants alone. In a dual-choice test, starved female wasps showed a significant preference for a B. rapa inflorescence over an inflorescence stem with flowers removed. The same female wasps showed no significant preference between aphid-infested and uninfested plants before they experienced these plants. However, female wasps that had been previously exposed to aphid-infested plants with honeydew significantly preferred aphid-infested plants to uninfested ones. These results suggest that parasitic wasps have cognitive abilities to find a variety of potential food sources and learn to recognize cues associated with foods. This flexible foraging behavior could allow them to increase their lifetime reproductive success.

Keywords:*Parasitoid, Food search, Floral scent, Herbivore-induced plant volatile, Cognition, Behavioral plasticity, Brassicaceae, nectar, Green peach aphid, honeydew*

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Effect of alternating temperature on life table parameters and predation capacity of phytoseiid spider mite predators

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Despite the large body of studies on the effect of constant temperatures on the biology of arthropods, the influence of varying temperatures remains largely understudied. In modern horticultural practice, however, considerable daily temperature variations are tolerated in the temperature integration approach. This energy saving strategy may have an influence on the development and performance of beneficial arthropods, including phytoseiid predatory mites. In the present study, life table parameters and predation rates of Phytoseiulus persimilis Athias-Henriot and Neoseiulus californicus McGregor were examined when fed with different life stages of the two-spotted spider mite Tetranychus urticae Koch at two constant temperatures (15°C and 20°C) and one alternating temperature (20°C/5° C). Rearing at the alternating temperature resulted in a significantly faster development compared to the corresponding mean temperature for both species. Combined with the higher overall reproductive performance this resulted in higher intrinsic rates of increase (rm). The rm increased from 0.086 to 0.114 for N. californicus and from 0.122 to 0.165 for P. persimilis for the alternating (20°C/5°C) and the corresponding mean temperature (15°C), respectively. When presented with T. urticae eggs, P. persimilis and N. californicus consumed more eggs at 20°C/5° C than at 15°C. However, when presented with nymphs of T. urticae no significant differences in predation rate were found between the alternating and corresponding mean temperature.

Keywords:*Phytoseiidae, temperature, biological control, Tetranychidae*

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Ecology

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Development, survival and reproduction of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) after its eggs exposure to high temperature stress

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The multicolored Asian ladybeetle, Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae), is a well known biocontrol agent. This study investigated the subsequently developmental, survival and reproductive traits of H. axyridis when its eggs were exposed to 25 (control), 37, 39 and 41°C for 1 hour, and then transferred to control condition. The hatching rate of eggs significantly differed among temperature treatments. The development, survival, weight, reproduction and longevity exhibited significant differences with temperature treatment and sex. The preimaginal development of females was not significant differences, while males exhibited dramatically differences with the increase of temperature. The survival rate of immature declined, while the adult fresh weight of both sexes markedly increased with the increase of temperature. Heat exposure of the eggs caused a subsequent reduction in longevity, oviposition period and reproduction, but the pre-oviposition period prolonged as the temperature increased. Interestingly, the fecundity showed increase from 37 to 39℃, though there was no significant difference; moreover, the latter oviposition period was shorter than that of the former. These may imply that the reproductive investment increased in higher level stressful environments, and the response of adult individuals could be linked to the experiences from early stages of the life history. Our findings firstly provide useful information for predicting population dynamics and distribution and also for better utilities of H. axyridis as biocontrol agent under variable environment.

Keywords:*Harmonia axyridis, heat shock, survival, longevity, reproduction*

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Ecology

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Climate warming increased fitness of the cicada, *Cryptotympana facialis*, by synchronizing its hatching time with the rainy season

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Climate change can disrupt the synchrony between organisms' requirements and resource availability, leading substantial changes in population and community structures. Here we address an impact of phenological shift by climate warming on fitness of Cryptotympana facialis (Hemiptera, Cicadidae). The population of this species drastically increased in Osaka, Japan in the late 20th century, and this change has been presumed to be related to climate warming. This species has a unique trait that hatching is induced in response to rain. As frequency of rain oscillates seasonally, phenological relationship between hatching time and the rainy season is supposed to influence the hatching success of the cicada. We showed that synchrony of the rainy season and hatching time could determine hatching success, by shifting the time of completion of embryonic development and supplying water at various intervals. We estimated the change in hatching time over the last eleven decades (1901?2009) based on the meteorological records and the temperature-dependent rate of C. facialis embryogenesis. Our estimation demonstrated that warming had advanced the hatching time by about two weeks during the 20th century, and improved synchrony with the rainy season in the late 20th century. The probability of hatching success was markedly variable and often very low before this synchronization, but became stably high thereafter. Therefore, the stabilizing effect of this synchrony on fitness was indispensable to the recent population increase of C. facialis.

Keywords:*climate change, hatching success, phenology, population increase, urban heat island*

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Ecology

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Macrodiplosis (Diptera: Cecidomyiidae) in Japan and Korea together with information on their ecological traits and gall induction on *Quercus* (Fagaceae)

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Leaf-margin fold galls on Quercus mongolica (Fagaceae) has been known to occur in Hokkaido, Japan. In the spring of 2011, many leaf-margin fold galls were found on the leaves of Q. serrata in southern Japan and Korea. Larval morphological features and the structure of galls indicated that these galls were induced by species of Macrodiplosis (Diptera: Cecidomyiidae). DNA sequencing data showed that the Macrodiplosis sp. 1 on Q. serrata was different from Macrodiplosis sp. 2 on Q. mongolica and closely related to a European M. roboris that is responsible for leaf-margin fold galls on Q. robur. All known Macrodiplosis species are univoltine and larvae develop quickly into third instars and quit the galls to drop down to the ground before summer. However, it is remarkable that Macrodiplosis sp. 1 passes through spring, summer and autumn as second instar and spins cocoons in the galls in November before leaf fall. In September 2011, we found leaf-margin fold galls of sp. 1 also on Q. mongolica in central Japan. At that time, the larvae already spun cocoons in the galls. The larvae seemed to develop more quickly in northern areas than in southern areas. The larvae of sp. 1 were parasitized by an unidentified ectoparasitoid through second to third larval stadia. We are now expecting adults emerging in the early spring of 2012.

Keywords:*Macrodiplosis, Cecidomyiidae, gall midge, Quercus*

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Ecology

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Effects of barley chromosome addition to wheat on feeding preference and nymphal development of the migratory locust *Locusta migratoria* (Orthoptera: Acrididae)

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The migratory locust Locusta migratoria exhibits density-dependent phase polyphenism and is potentially one of the most destructive agricultural pests worldwide. The locust feeds on various Poaceae such as wheat, but does not eat barley. Identification of the barley genes inhibiting feeding by the locust is useful for developing resistant crops. Using six barley chromosome disomic addition lines of wheat (2H-7H), we investigate the effects of barley chromosome addition to wheat on feeding preference and nymphal development of L. migratoria. Feeding preference by locust hatchlings was investigated by choice tests in which wheat and one of the chromosome addition lines were presented to locust hatchlings for 24 hours. The mean amount of wheat consumed by hatchlings (control) was significantly larger than that of 2H, 5H, and 6H fed-locusts. Growth performance of the hatchlings fed with wheat, barley, or 2H-7H was assessed by examining their survival rate and developmental period. The duration of the first instar for hatchlings given 2H or barley was significantly longer than that for those given wheat or the other addition lines. Based on these results, we conclude that the barley genes involved in the inhibition of feeding by locusts are located on barley chromosomes 2, 5, and 6, and those responsible for slow development of the locust on barley chromosome 2.

Keywords:*Locusta migratoria*

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Ecology

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Male-killing *Wolbachia* and mitochondrial selective sweep in a migratory African insect

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Numerous recent studies have shown that resident symbiotic microorganisms of insects play a fundamental role in host ecology and evolution. Wolbachia pipiensis infections and the co-inherited marker, mtDNA, were examined as a method to explore the evolutionary history and population biology of the agricultural pest, African armyworm (Spodoptera exempta), a highly migratory and destructive species in sub-Saharan Africa. Three strains of Wolbachia were detected, one of which was found to be a male-killer, and genetically identical to a previously described Wolbachia from the Blue Moon Butterfly, Hypolimnas bolina. Partial sequencing of the mitochondrial COI gene from 164 individual armyworm of known infection status revealed 17 different haplotypes. There was a strong association between Wolbachia infection status and mtDNA haplotype, with only a single haplotype, haplo1 (90.2% prevalence), harbouring the endosymbiont. This indicates that Wolbachia may be driving a selective sweep on armyworm haplotype diversity. Despite very strong biological and molecular evidence (including from nuclear 28S gene markers) that the samples represent a single species (S. exempta), the 17 haplotypes did not fall into a monophyletic clade within the Spodoptera genus; with six haplotypes (2 each from 3 geographically separate populations) differing by >11% in their nucleotide sequence to the other eleven. This has clear implications for the use of mtDNA as neutral genetic markers in insects, and also demonstrates the impact of Wolbachia infections on host evolutionary genetics.

Keywords:*Wolbachia, Cytochrome Oxidase I, selective sweep, Spodoptera exempta, African armyworm*

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Ecology

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Microsatellites reveal a strong subdivision of genetic structure in Chinese populations of the mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Background: Two colour forms of the two-spotted spider mite (Tetranychus urticae Koch) coexist in China: a red (carmine) form, which is considered to be native and a green form which is considered to be invasive. The population genetic diversity and population genetic structure of this organism were unclear in China, and there is a controversy over whether they constitute distinct species. To address these issues, we genotyped a total of 1,055 individuals from 18 red populations and 7 green populations in China using eight microsatellite loci. Results: We identified 109 alleles. We found a highly significant genetic differentiation among the 25 populations (global FST=0.506) and a low genetic diversity in each population. In addition, genetic diversity of the red form mites was found to be higher than the green form. Pearson correlations between statistics of variation (AR and HE) and geographic co-ordinates (latitude and longitude) showed that the genetic diversity of the red form was correlated with latitude. Using Bayesian clustering, we divided the Chinese mite populations into five clades which were well congruent with their geographic distributions. Conclusions: Spider mites possess low levels of genetic diversity, limit gene flow between populations and significant and IBD (isolation by distance) effect. These factors in turn contribute to the strong subdivision of genetic structure. In addition, population genetic structure results don't support the separation of the two forms of spider mite into two species. The morphological differences between the two forms of mites may be a result of epigenetic effects.

Keywords:*two-spotted spider mite, green form, red form, genetic diversity, null alleles*

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Invasion genetics of the western flower thrips in China: Evidence for genetic bottleneck, hybridization and bridgehead effect

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The western flower thrips, Frankliniella occidentalis (Pergande), is an invasive species and the most economically important pest within the insect order Thysanoptera. F. occidentalis was initially detected in Kunming in southwestern China in 2000 and since then it has rapidly invaded several other localities in China where it has greatly damaged greenhouse vegetables and ornamental crops. Controlling this invasive pest in China requires an understanding of its genetic makeup and migration patterns. Using the mitochondrial COI gene and 10 microsatellites, we investigated the genetic structure and the routes of range expansion of 14 F. occidentalis populations in China. Both the mitochondrial and microsatellite data revealed that the genetic diversity of F. occidentalis of the Chinese populations is lower than that in its native range. Two previously reported cryptic species (or ecotypes) were found in the study. The divergence in the mitochondrial COI of two Chinese cryptic species (or ecotypes) was about 3.3% but they cannot be distinguished by nuclear markers. Hybridization might produce such substantial mitochondrial-nuclear discordance. Furthermore, we found low genetic differentiation (global FST = 0.043, P < 0.001) among all the populations and strong evidence for gene flow, especially from the three southwestern populations (Baoshan, Dali and Kunming) to the other Chinese populations. The directional gene flow was further supported by the higher genetic diversity of these three southwestern populations. Thus, quarantine and management of F. occidentalis should focus on preventing it from spreading from the putative source populations to other parts of China.

Keywords:*western flower thrips, invasion genetics, microsatellites*

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Seasonal trends and physiological condition of the green stink bugs, *Nezara viridula* and *N. antennata* (Heteroptera: Pentatomidae) caught in the light trap

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The southern green stink bug, Nezara viridula (L.) (Heteroptera: Pentatomidae) is a serious agricultural pest throughout the world. In recent years, N. viridula has been expanding its range northward in Japan, probably due to the global warming. To develop the monitoring system of N. viridula, we investigated the seasonal and temporal trends of attraction to the light trap, and also compared the attraction with its allied congener, the oriental green stink bug, Nezara antennata Scott, which is widely distributed in Japan. The sex ratio of the N. viridula adults caught in the light trap was highly female-biased, yet almost equal numbers of N. antennata adults were caught throughout the experimental period. After September, still a large number of N. viridula adults were caught in the light trap, while few number of N. antennata were caught. During the scotophase, N. viridula were caught mainly before midnight, while N. antennata were caught mostly after midnight. These results suggest that the response to the light or the flying activity during the scotophase varied between these two species. Physiological condition, especially ovarian development of females, fluctuated during the season, and the possibility of predicting the changes of seasonal history of these Nezara species is discussed.

Keywords:*physiological condition, light trap, monitoring, Nezara viridula, global warming*

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Ecology

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Evolutionary convergence of leafy moth and butterfly wing patterns to establish functional modules

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Camouflage is adaptive morphological traits, resemblance to natural objects including leaves and lichens. Such mimetic patterns are typically composed of several components, and seem to achieve a specific integration to make an adaptive performance. However, how the mimetic leafy patterns are integrated has been little investigated. Here, we developed a novel method, "morphometrical network analysis", to reveal variational modules of leafy moth/butterfly wing patterns. As a model organism, we used a noctuid moth, Oraesia excavata, and a nymphalid butterfly, Kallima inachus, which closely resemble leaves with a leaf venation patterning. According to phylogenetic analysis, these two species independently evolved their leafy patterns. Our quantitative analysis indicated that in both species the constituent components of the leafy patterns are tightly coupled as modules, each of which corresponds to each component of the leaf venation pattern (i.e., the main, right-hand, and left-hand venation patterns). Interestingly, comparative morphological analysis (based on ground plan of lepidopteran wing patterns) showed that two leafy patterns are composed of the same set of homologous elements, but the correspondent leaf veneation components are established by using different elements. To put it all together, we concluded that the modules corresponding to the leaf venation components were emerged through convergence evolution, strongly suggesting that modules detected here are adaptive consequences in response to natural selection. Our results may suggest that the modules likely improve the visual trick of leafy camouflage.

Keywords:*Lepidopteran wing patterns, Camouflage, Evolution, Modules, Morphometrics*

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Ecology

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Brood parasitism in two species of spider wasps (Hymenoptera: Pompilidae: Dipogon), with notes on a novel reproductive strategy

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Wasps of brood parasitic species do not themselves hunt, but rather exploit prey caught by other individuals. These species either lay an egg on paralyzed prey being transported by another species (type A), or else they sneak into the nests of other species and lay their eggs on or near the stored prey (type B). Hatched larvae of the parasite then consume the usurped prey, taking the place of the host larvae. The purpose of this study was to verify the existence of type B brood parasitism in the two pompilid species, Dipogon iwatai and D. nagasei, based on nesting records. To observe nest contents, we used the trap-nest technique, which was indispensable for this study because these species are rare and their behavior is difficult to observe in the field. As a result of our observations, it was found that D. nagasei brood parasitized other species of Dipogon, whereas D. iwatai brood parasitized Auplopus carbonarius, together with several species of Dipogon. Both brood parasitic species are with the subgenus Nipponodipogon, whereas the Dipogon hosts are in the subgenus Deuteragenia. Comparison of their ecological and morphological features revealed that brood parasitism in D. nagasei is considerably more derived than in D. iwatai. Of particular note is the fact that in D. nagasei the female routinely lays up to five eggs on a single host spider, all of which develop into adult wasps without larval cannibalism; almost all Pompilidae previously studied lay only one egg on a host spider.

Keywords:*brood parasitism, Deuteragenia, Dipogon, Nipponodipogon, Pompilidae, spider wasps, trap nest*

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Maternal oviposition site selection in the stinkbug *Urostylis westwoodi* (Hemiptera: Urostylidae).

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In many organisms, development and success of their offspring is strongly affected by maternal oviposition strategies. Plant-sucking stinkbugs (Hemiptera: Pentatomomorpha) adopt various oviposition strategies, such as difference in clutch sizes and a variety of maternal cares. Stinkbugs of the family Urostylidae are distributed in southern and eastern Asia, with five species in Japan. All three Japanese Urostylis species lay their eggs on the trunk of Quercus trees toward the end of autumn, and after about three months, the eggs hatch in the end of winter. Of special note is that their egg masses are covered with a huge amount of jelly-like substance secreted by mother insect during oviposition. This substance is the sole food source for nymphs, on which they grow to 3rd instar toward early spring. Here we report a field survey of oviposition patterns by females of Urostylis westwoodi. Our observational data revealed that egg masses of U. westwoodi tend to be laid on the northern side of the tree trunks. The orientational differences of oviposition sites affected the sun-shade patterns, and consequently thermal patterns the egg masses experienced. Namely, in the egg masses oriented to the south, the eggs tended to hatch earlier and the nymphs tended to grow faster. These results suggest that the northward ovipositional site selection by U. westwoodi females is not for maximal nymphal growth, but is likely for avoidance of desiccation of the jelly-like substance and also for adjustment of the nymphal development to the timing of plant bud opening.

Keywords:*Heteroptera, ovipositional site selection, overwinter, orientation*

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Ecology

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Phototactic behavior and visual function between nymphs and alates of a termite, *Zootermopsis nevadensis* (Hagen)

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Most castes of termites usually avoid bright light, but alates do not. The alates fly out from their nests in the daytime, and are attracted to street lights at night. In this study, we used the Dampwood termite, Zootermopsis nevadensis, which swarms during daytime, to investigate the differences in phototactic behavior between nymphs and alates. We also determined the spectral sensitivity of compound eyes based on electroretinogram (ERG) to clarify the relationship between the behavior and visual function. In behavioral assays, nymphs walked away from the light source of 350-375 nm (UV) and 400-425 nm (violet?blue). However, alates were strongly attracted to the area irradiated with the same lights, and even flied toward the light source repeatedly. Neither nymphs nor alates responded to 620-700 nm (red) light. The spectral sensitivity of nymphs had the primary peak at 520 nm (green) and the secondary peak at 360 nm (UV). In alates, we found the primary peak at 360 nm and the secondary peak at 520 nm. No sensitivity was detected in 620-700 nm neither nymphs nor alates. These results indicate that relationship between the phototactic behavior and spectral sensitivity in both nymphs and alates are strong in the UV and weak in the green wavelength region.

Keywords:*Termite, Phototactic behavior, Visual function*

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Ecology

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Overview of pheromone diversity in hawk moths based on receptor and behavioral responses

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Sphingidae is a large group in Bombicoidea with ca. 1,400 species and also a well-studied group of insects. However, the sex pheromones of this family have been identified in only a few species. We investigated the sex pheromone systems of hawk moths and found diversity in sex pheromone components. In the present study, we surveyed male responses to the sex pheromone components at both levels of odorant receptor and behavior. Insects were collected from the campuses of Tottori University and University of Tsukuba and reared under laboratory conditions. Sex pheromone components were determined by conventional methods. Pheromone candidate compounds were clarified by gas chromatography-electroantennographic detection (GC-EAD). Chemical structures of the candidate compounds were determined by GC-mass spectrometry (GC-MS) and GC analysis. In pheromone gland extracts, 10,12-hexadecadienals, 11-hexadecenals and 9,11-pentadecadienals were found to be sex pheromone candidates from 14 species of hawk moths. Behavioral responses of male moths to the sex pheromones were examined by field trap tests with synthetic lures. Male moths were exclusively attracted to the natural components of conspecific females. GC-EAD analysis was also conducted to determine the spectral ranges and sensitivities of male antennal pheromone receptors to these compounds. Diversity of sex pheromones in hawk moths will be discussed from the perspectives of peripheral receptor and behavioral responses.

Keywords:*Hawk moth, Sex pheromone, Bombykal*

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Host specificity of Poropoea morimotoi and reasons for this specificity

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Attelabid beetle females roll leaves to provide food for their brood, and some species cut off the leaf rolls. Poropoea morimotoi Hirose 1963 (Hymenoptera: Trichogrammatidae) is the most common egg parasitoid of attelabid beetles in Japan. Female wasps have a long ovipositor to lay eggs in the beetles' egg in the leaf rolls. Eleven species of attelabid beetles are recorded as hosts of P. morimotoi. However, there are no studies on host specificity in a biotic community of attelabid beetle species. In this study, we examined the parasitic status of P. morimotoi in 2 biotic communities that include 6 species of beetles throughout a year. We demonstrated that P. morimotoi can successfully parasitize certain species of the beetles. In addition, our results suggested two reasons for this host specificity. One is the length of the ovipositor of the wasps makes it so they can lay eggs only on species in small leaf rolls and cannot lay eggs on species in large leaf rolls. Second is the influence of beetle behavior. Wasps mainly parasitized suspended leaf rolls so cutting off a leaf roll may be a barrier for wasps to lay eggs. In conclusion, wasp host specificity may be determined by differences in leaf roll size and leaf roll cutting behavior of attelabid beetles.

Keywords:*parasitoid-hosts interaction, attelabid beetle, leaf-roll, egg parasitoid*

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Strepsipteran parasites (Strepsiptera: Stylopidae) control their host's pollination behavior.

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Strepsiptera, known as 'twisted-winged parasites', is an insect order with nine families making up about 600 species. They are parasites of other insects. The male is the winged, dispersal stage; females are apterous, larviform, and viviparous and permanently reside within their host in all but the most basal family. The sclerotized head end (cephalotheca) of larviform females typically protrudes slightly from between host tergites, copulating with a male and exiting many small first instar larvae through a brood-canal opening on the cephalothorax. Usually parasitized hosts lose their fecundity. First instar larvae of Stylopidae strepsipterans show specialized forms for phoresy. The larvae disperse by phoresy through flowers to reach and parasitize eggs and larvae of host bees. Parasitized host bees lose their fecundity and nest building instincts. Though bees visit flowers and collect pollen and nectar, they feed only nectar. Adult bees collect pollen as food for larvae, so parasitized bees that don't build their nests need not collect pollen. When the parasitized bees visit flowers that provide only pollen as reward, the bees gain nothing by visiting flowers. While it is efficient for strepsipterans to infest new host by they control parasitized bees to flowers where nests building non-stylopized bees visit frequently. In this study, we observed the pollination behavior of stylopized bees and revealed that strepsipteran parasites control the pollination behavior of their host bees.

Keywords:*host control, pollinator, Halictoxenos, Lasioglossum*

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Does host plant regrowth mediate coexistence of herbivorous insects?

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The Competitive Exclusion Principle predicts that two species sharing a same niche cannot coexist. However, many specialist herbivorous insects coexist despite of sharing their host plant in the field. There is increasing evidence that interspecific interactions among herbivorous insects are mainly mediated by food plant responses. Such indirect interaction may mediate coexistence of herbivorous insects. However, information about long-term outcomes (i.e. coexistence or exclusion) of such indirect interactions is still lacking. In 2011, we studied an exotic butterfly Sericinus montela and a native butterfly Atrophaneura alcinous sharing the same host plant Aristolochia debilis. Only these butterflies can feed on A. debilis, and they have no specific natural enemies due to defensive chemicals sequestered from A. debilis in their body tissue. In the field, leaves of A. deibilis were often consumed completely by these caterpillars, but they can regrow after some time and then were eaten again by other caterpillars. These results suggested that these two butterflies are coexisting not because their resources are abundant, and that such plant regrowth may mitigate the competitive interaction between the two butterfly species. We will show whether the plant regrowth promotes coexistence of the two butterfly species.

Keywords:*species coexistence, interspecific interaction, plant regrowth, herbivorous insects, exotic species*

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Recovery of aquatic insects in a pond of Fukushima, Japan after The Great North Eastern Japan Earthquake & Tsunami

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The Great North Eastern Japan Earthquake & tsunami on March 11TH, 2011, inundated the coastlines of Fukushima prefecture. This force damaged many human lives and biodiversity. In addition to this, the habitat of the organisms was exposed to radiation from the accident at the Fukushima No. 1 nuclear power plant. Therefore investigation of natural environment is not progressing in the coastlines of the Fukushima Prefecture. We investigated a pond near the coastline of Fukushima Prefecture. This fresh water pond (about 260 m2 , about 60 cm deep) was in the damaged area of tsunami, Matsukawaura, Soma city. Twenty-three days after the tsunami, the salinity of this pond was 1.25% (April 3, 2011), and it decreased to 0.08% after a year of tsunami (March 19, 2012). Aquatic insects of 20 species (4 order 8 family) were found in this pond from 2008 to 2010. Aquatic insects were very few in numbers after the tsunami. Then the number of insects increased gradually, and aquatic insects of 25 species (4 order 8 family) were found in one year. The number of larva of Aeschnidae decreased. On the other hand, 10 species (Coleoptera: 6 species, Hemiptera: 4 species) were newly recorded. Out of the 10 species, 3 species, Cymatia apparens, Anisops ogasawarensis, Eretes sticticus, are especially high in numbers. The insect fauna of this pond changed dramatically after the tsunami.

Keywords:*aquatic insect, tsunami, recovery, Fukushima*

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Overwintering strategy of the egg parasitoid wasp *Trissolcus plautiae*: Enhanced cold tolerance and reduced reproductive ability in response to photoperiod

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The egg parasitoid wasp Trissolcus plautiae (Watanabe) (Hymenoptera: Scelionidae) is the most important natural enemy of Plautia stali Scott (Hemiptera: Pentatomidae), a serious pest causing fruit damage in Japan. Females of this wasp overwinter maintaining their reproductive activity without being induced into reproductive diapause. The wasp's overwintering ability depends on the emergence date. Observations under natural conditions in the Kanto region Japan showed a sharply reduced survival after November for females that ended development before mid-August, whereas not for females that emerged after mid-August. The light regime experienced in the larval stage has a remarkable effect on the cold tolerance of adults. Females reared under a long photoperiod of 16L:8D had a low survival rate at -5 ºC or below, and survived less than one month even at 5 ºC, whereas those reared under a short light period of 10L:14D maintained a high survival rate over two weeks at -7.5 ºC. Although experimental combinations of temperature and photoperiod during development did not lead females to reproductive diapause, the number of eggs in ovaries was significantly reduced by a short photoperiod. Science its host has a large variation in seasonal occurrence, this overwintering strategy of improved cold tolerance and holding fewer matured eggs is likely an adaptation to the unpredictable availability of resources in time and space.

Keywords:*natural enemy, egg parasitoid wasp, Trissolcus, Pentatomidae, overwintering, non-diapause, cold hardiness, photoperiod*

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Ecology

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Pupae of a rhinoceros beetle produce deceptive vibratory signals to protect themselves against conspecific larvae

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It is argued that animal signals may have evolved so as to manipulate the response of receivers in a way that increases the fitness of the signallers. In deceptive communication, receivers incur costs by responding to the false signals. The larvae of Japanese rhinoceros beetle Trypoxylus dichotoma (Coleoptera, Scarabidae, Dynastinae) construct their own pupal cells to ensure normal pupation and eclosion. These cells are fragile and subject to damage from burrowing larvae because this species shows a highly aggregated distribution in the field. Recently, we reported that pupae of this species produce vibratory signals to deter conspecific larvae, thereby protecting themselves (Kojima et al. 2012 Behav Ecol Sociobiol). In the present study, monitoring of vibrations associated with larval movement revealed that T. dichotoma larvae remained motionless (freeze response) for ca. 10 min when pupal vibratory signals were played back transiently. Furthermore, pupal signals of T. dichotoma elicited a freeze response in three other scarabaeid species from Cetoniinae and Rutelinae, whose pupae do not produce vibratory signals. This indicates that the freeze response to certain types of vibration evolved before the divergence of these species, and has been evolutionarily conserved presumably because of the fitness advantage in avoiding predators. Pupae of T. dichotoma have probably exploited preexisting anti-predator responses of conspecific larvae to protect themselves by emitting deceptive vibratory signals.

Keywords:*communication, deception, scarab beetle*

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Community-wide impacts of foliar herbivory on flower visitors

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It has been accepted that foliar herbivory has an indirect and negative impacts on plant reproductive success by reducing pollinator visitation. However, indirect effects of early-season foliar herbivory on floral visitation of non-pollinators such as florivores and predators, which may also influence pollinator visitation, are poorly appreciated. To fully understand the effects of foliar herbivory on plant reproductive success, we need to explore its impacts on overall community structure of flower visitors including non-pollinators. In this study, we examined how foliar herbivory can influence community structure of floral visitors. To address this question, we conducted an inoculation experiment using lace bug (Corythucha marmorata), which is a predominant herbivorous insect attacking Solidago altissima in Japan. In damaged plants, plant height, flower numbers and branches with flowers significantly decreased compared to undamaged plants. Also, flowering phenology was significantly delayed in damaged plants. Relative pollinator abundance except for flies did not differ between damaged and undamaged plants. The number of non-pollinators, including spiders, stink bugs, caterpillars, and grasshoppers decreased on damaged plants. The pollinator visitation was positively correlated with flower number. However, non-pollinator visitation was not related to the flower number, suggesting that changes in the floral quality may affect non-pollinators visitation. We found that overall community structure of flower visitors significantly differed between damaged and undamaged plants.

Keywords:*pollination herbivory florivory*

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Ecology

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Genetic evaluation of the bumblebee (Hymenoptera, Apidae), *Bombus deurteronymus maruhanabachi*, endangered species of semi-natural grasslands in Nagano, Japan

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The bumblebee Bombus deuteonymus deuteronyms (Bdd) adapts to grasslands in East Asia, including Hokkaido, Japan. The subspecies B. d. maruhanabachi (Bdm) is endemic to Honshu island of Japan and shows close connection to semi-natural grasslands, which is a type of grassland that has been maintained by human activities. This grassland is considered one of the refugia for many plant and insect species that had immigrated to Japan from Asian continent probably through Korean Peninsula. Rapid decline of semi-natural grasslands during the 20th century in Japan is deeply concerned to reduction and fragmentation of habitats for grassland species. The present study aims to characterize genetic diversity of Bdm distributed in Nagano Prefecture, Japan by comparing with Bdd populations from Primorsky Territory, Russia and Hokkaido, and also two other species of Bombus (B. hypnorum and B. hypocrita) living in Nagano. Ten loci of microsatellite DNA were genotyped and population genetic parameters were calculated. Based on comparison of allelic richness and mean heterozygosity, genetic diversity of the Nagano population of Bdm was lower than the Primorsky population of Bdd, and also lower than two other Bombus species. The results of AMOVA showed no genetic structure in Bdm, but pairwise population differentiation analysis revealed significant differentiation between some local populations in Nagano. Mean population relatedness was higher in Bdm population comparing to two other species in Nagano. These results suggest a sign of degradation of habitat environments for Bdm and other grassland species.

Keywords:*Bombus deuteronymus, Bumblebee, genetic diversity, semi-natural grassland, microsatellite DNA*

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Ecology

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Oviposition behavior of rice leaf bug *Trigonotylus caelestialium* (Kirkaldy) and sorghum plant bug *Stenotus rubrovittatus* (Matsumura)

Atsuhiko Nagasawa
Tohoku University, Japan

The rice leaf bug Trigonotylus caelestialium and sorghum plant bug Stenotus rubrovittatus are important pests of rice plants in Japan. These insects cause formation of discolored grains known as pecky rice. Further, the main hosts of these insects are Poaceae plants. Here, I investigated suitable plant species for studying the oviposition behavior of these mirid bugs. Adult mirid bugs oviposited on various Poaceae and Cyperaceae. Nymphs of T. caelestialium emerged from both spikelets and leaf sheaths, whereas those of S. rubrovittatus emerged almost exclusively from the spikelets. Eggs of T. caelestialium were also observed on both spikelets and leaf sheaths, whereas those of S. rubrovittatus were observed only on the spikelets of some plants. These indicate that the preference of oviposition sites is different between T. caelestialium and S. rubrovittatus. To understand the difference between preference of oviposition sites of T. caelestialium and S. rubrovittatus, I observed the following oviposition behavior of these mirid bugs: The adult mirid bugs always inserted their stylets into the spikelets before oviposition. After pulling the stylet out of the spikelet, the adult inserts its ovipositor into the same place. This behavior indicates that the adult bug recognizes the oviposition site using its stylet. I observed T. caelestialium show such searching behavior on leaf sheaths using its stylet, whereas no such behavior was shown by S. rubrovittatus. Thus, these 2 bugs might recognize oviposition sites before using their stylets.

Keywords:*oviposition behavior, Trigonotylus caelestialium, Stenotus rubrovittatus, Poaceae, stylet, pecky rice*

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Ecology

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Ability of making trench determines host plant range of cucurbit feeding insects

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Cucurbit feeding insects, such as Aulacophora and Epilachna beetles, make trench on their cucurbit host plant leaves before feeding on it, protecting the feeding site from sap exudation. Although these insect species have different host plant range each, they feed on non-host cucurbit plants when provide the detached leaves from the whole plant body. In laboratory experiments using whole plant body and the detached leaves of pumpkin and Trichosanthes plants, Epilachna admirabilis (hereafter Ea, host plant: Trichosanthes) fed on the detached leaves of pumpkin, a non-host plant, without making trench; however, Ea did not make a trench on the whole body leaves of pumpkin, nor they fed on it. Aulacophora indica (hereafter Ae, host plant: pumpkin and Trichosanthes etc.) fed on both whole body and the detached leaves of Trichosanthes as well as pumpkin. Observation by a stereoscopic microscope revealed that bite marks and sap exudation remained on surface of the leaves of whole pumpkin body by Ea. This suggests that capability of trench makes the host plant range: Ae has high ability of making trench to prevent sap from exuding and Ea do not make a trench effectively to prevent sap from exuding from whole pumpkin plants. Further feeding mechanisms and factors influencing the host plant selection by Ae, Ea and other cucurbit feeding insects will be discussed.

Keywords:*Trench, Host plant selection, cucurbit feeding insects,*

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Fatty acid-amino acid conjugates diversification in Lepidopteran caterpillars

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Fatty acid amino acid conjugates (FACs) have been found in noctuid as well as sphingid caterpillar oral secretions; in particular, volicitin [N-(17-hydroxylinolenoyl)-L-glutamine] and its biochemical precursor, N-linolenoyl-L-glutamine, are known elicitors of induced volatile emissions in corn plants. These induced volatiles, in turn, attract natural enemies of the caterpillars. In a previous study, we showed that N-linolenoyl-L-glutamine in larval Spodoptera litura plays an important role in nitrogen assimilation which might be an explanation for caterpillars synthesizing FACs despite an increased risk of attracting natural enemies. However, the presence of FACs in lepidopteran species outside these families of agricultural interest is not well known. We conducted FAC screening of 29 lepidopteran species, and found them in 19 of these species. Thus, FACs are commonly synthesized through a broad range of lepidopteran caterpillars. Since all FAC-containing species had N-linolenoyl-L-glutamine and/or N-linoleoyl-L-glutamine in common, and the evolutionarily earliest species among them had only these two FACs, these glutamine conjugates might be the evolutionarily older FACs. Furthermore, some species had glutamic acid conjugates, and some had hydroxylated FACs. Comparing the diversity of FACs with lepidopteran phylogeny indicates that glutamic acid conjugates can be synthesized by relatively primitive species, while hydroxylation of fatty acids is limited mostly to larger and more developed macrolepidopteran species.

Keywords:*Lepidoptera*

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Ecology

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Measuring trunk volatiles of spruce (*Picea abies*) using an optimized trunk enclosure

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Volatiles (Volatile Organic Compounds, VOCs) play an important role in host plant finding and host plant choice of insects. In contrast to volatiles released by leaves, the release of volatiles from tree trunks and their impact on xylophagous insects is not well known yet. Trunk volatiles are expected to be of significant importance in the host finding process of wood living insects. This study presents an improved method for the sampling of trunk volatiles with reduced cost and time expenditure. The method is based on a flexible PET enclosure and a volatile trapping unit. The flexible PET foil is used to constitute an isolated space around a cylindrical part of the trunk. The volatiles released by the trunk are constantly extracted for two hours in an open loop mode. The samples are trapped on Tenax traps and CLSA-traps. These samples are desorbed by a Thermal Desorption System (TDS) and by chemo-desorption, respectively. The chemical analysis is performed by a gas chromatograph with a mass spectrometric detector (GC-MS).

Keywords:*VOC, spruce, trunk, xylophagous beetles*

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Ecology

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Genetic structure of *Planococcus ficus* (Hemiptera: Pseudococidae) from vineyards in Egypt.

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Planococcus ficus is an important pest in vineyards worldwide. Despite its economic importance, the taxonomic status of its populations is still unclear. For example, populations from Egypt and Israel currently referred to as P. ficus were formerly split into two species then synonymized by Cox & Ben-Dov in 1986. Recent DNA barcoding results in Egypt revealed substantial genetic variations among samples at several DNA markers, hence calling for further investigations of the population genetic structure. In this work, we investigated the genetic structure of P. ficus populations in Egyptian vineyards. Populations from 5 regions were collected and subjected to DNA sequencing at the mitochondrial gene Cytochrome oxidase I. The population genetic diversity and genetic structure were studied by estimating standard diversity and Fst indices, and performing an analysis of molecular variance. Fifteen COI haplotypes were observed. Significant genetic differentiation was detected among populations with Fst ranging from 0.471 to 1. Among regions genetic differentiation was moderate with Fst values from 0.076 to 0.349. The Mantel test showed no significant correlation between genetic and geographical distances. Our results do not provide evidence of the occurrence of more than one taxon. Rather, the observed high genetic diversity suggests that Egypt is part of the native region of one species. The results also suggest that the population genetic structure is mainly driven by genetic drift. This is likely the consequence of repeated demographic bottlenecks caused by pesticide treatments; each population probably corresponds to the progeny of few surviving individuals from the previous generation.

Keywords:*Mealybug, Planococcus ficus, Planococcus vitis, Polymorphism, Genetic Structure*

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Ecology

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Behavioral response of *Harmonia axyridis* towards their footprints according to their physiological state

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In order to survive cold, the multicolored Asian ladybeetle, Harmonia axyridis (Pallas), aggregates inside dwellings during winter. It has been recently highlighted that overwintering H. axyridis individuals lay an area marking while walking, which is used by conspecifics to locate aggregation sites. These footprints are made-up of hydrocarbons, comprising both saturated and unsaturated homologues. However, it has not been demonstrated whether this "following area marking" behavior is specific to the overwintering individuals. The work presented herein was oriented to the study of the chemical evolution of these footprints according to the physiological state of H. axyridis. Monthly GC-MS analyses revealed that the area marking contained a greater amount of di-unsaturated compounds when laid by overwintering ladybeetles, suggesting the great importance of these chemicals in the ladybeetles aggregation process. In the second instance, behavioral investigations conducted in a Y-shaped glass tube were performed to assess (1) the evolution of H. axyridis behavior towards their footprints and (2) whether this behavioral modification is due to an evolution of the ladybeetles sensitivity or rather to an evolution of the area marking attractiveness. The results revealed that only the overwintering individuals follow their area marking, and that this behavior is linked to the ladybeetle physiological state rather than to the chemical profile of the marking biomolecules.

Keywords:*ladybird, behaviour, chemical ecology*

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Ecology

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Characterization of *Harmonia axyridis* (Pallas) overwintering sites

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Originally introduced as a biological control agent, the multicoloured Asian ladybeetle, Harmonia axyridis (Pallas), has become an invasive pest throughout Europe and North America in the last few years. Although its effectiveness to control aphid and coccid populations was impressive, some negative impacts appeared rapidly, notably on human health. Indeed, to protect themselves from cold temperatures, H. axyridis individuals move inside dwellings and buildings and form large aggregations in concealed portions of structures to overwinter. The aggregating beetles are responsible for some annoyances due to, on one hand, the number of individuals inside homes and, on the other hand, the hemolymph secretions they release when they are disturbed, which can cause allergic reactions. In order to highlight the specific features of infested houses, we investigated a large number of overwintering sites in Wallonia between 2007 and 2011. These sites were characterized through a survey sent to homeowners confronted to invasion problems. This survey was mainly focused on a general description of the infested house (type, colour, infested floor(s), building material), the orientation of the colonized rooms and the position of the beetles' cluster. The collected data indicate that H. axyridis preferentially selects isolated brick houses with red or white fronts to take shelter. Aggregations are mostly located at the first floor, essentially inside south or west oriented rooms. Furthermore, ladybeetles generally gathered into wooden windows frames facing south or west, and to a lesser extent, in the upper corners of walls presenting the same orientation. All these results

Keywords:*ladyvbird, ecology, behaviour*

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Ecology

P3

The effects of organic and conventional management strategies on biodiversity and ecosystem functioning in paddy fields

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Recently, conventional farming has caused serious ecological problems such as loss of biodiversity, although organic farming may be beneficial for conserving biodiversity and improving the agroecosystem. There are several studies suggesting that biodiversity in organic was higher than conventional farming, however, it still remains unclear how these management ways influence on the ecosystem processes. We expected that different nutrient in the soil between the two management ways might influence the ecosystem processes and affect biodiversity. Field researches were conducted to understand the biodiversity in organic and conventional farming. In general, biodiversity between organic and conventional farming was not significantly different. In addition, to understand ecosystem processes, experiments regarding feeding behaviour of the most abundance herbivore such as grasshopper were conducted in a laboratory. Feeding preference and performance of the grasshopper increased when rice plants in conventional farming were fed as food, because soil nutrient such as Nitrogen in the conventional farming was higher than organic farming. These results suggested that the method of management would influence on soil nutrient and give different impacts on biodiversity in a paddy field.

Keywords:*conventional farming, organic farming, biodiversity, soil nutrient, paddy fields,*

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Ecology

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Electrophysiological and behavioural responses of *Thanatophilus sinuatus* Fabricius (Coleoptera: Silphidae) to selected cadaveric odors

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The sense of smell or olfaction is crucial for insects and carrion insects are not exception to this, they have adapted their olfactory system over millions of years to specific cadaveric chemicals. Forensic entomologists often raise the hypothesis that these cadaveric compounds regulate the necrophagous insect's behavior and are responsible to the attraction of carrion insects to the corpses, including Silphidae. Nevertheless, it is not known which chemical cues are involved in the attractiveness of the carcass. So far, little information is available on the chemical ecology of carrion beetles, particularly concerning the subfamily of Silphinae. Silphinae are frequently found on vertebrate carcasses and may be present in great numbers. Thanatophilus sinuatus F. is frequently found in Europe on vertebrate carcasses. The biological role of selected cadaveric volatile organic compounds including: dimethyldisulfide (DMDS), butan-1-ol, n-butanoic acid, indole, phenol, p-cresol, putrescine, and cadaverine on the silphine species, Thanatophilus sinuatus, was investigated by using both electrophysiological and behavioural techniques. Among the tested cadaveric compounds, butan-1-ol and DMDS elicited the strongest EAG from both T. sinuatus male and female antennae. In a two-arm olfactometer, males and females were significantly attracted to dimethyldisulfide (DMDS) for both tested doses, whereas only males were attracted to p-cresol at 100 ng. Putrescine was repellent to males at the dose of 1 µg.

Keywords:*behaviour, forensic, chemicalecology*

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Ecology

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Volatile organic compounds released by barley roots attract wireworms

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Wireworms are the soil dwelling larvae of click beetles and are pests of many crops worldwide. Alternatives to insecticide treatments are needed in order to develop integrated management strategies. Our work consists in elucidating the role of barley root-emitted volatile organic compounds on the orientation behaviour of Agriotes sordidus wireworms. Using a dual choice olfactometer we have evaluated the attractiveness of a variety of baits ranging from barley roots themselves to isolated root-emitted volatile organic compounds. Wireworms were significantly attracted towards most of the tested baits. Our results should be taken into account in varietal selection, in crop rotation, or in trapping systems aiming at the reduction of the populations of this pest.

Keywords:*behaviour, up below ground, chemical ecology*

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Ecology

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AQUATIC INSECTS IN ALPINE LAKES (SLOVAKIA, EUROPE): A SMALL-SCALE POPULATION-GENETIC STUDY

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High mountain lakes of glacial origin belong to the most remote and undisturbed aquatic environments over the world, and their recovery from anthropogenic pollution, or reactions to climate change are hot topics of freshwater research. Population genetic studies of alpine lakes inhabitants mostly describe genetic patterns in large scale (between mountain ranges). However, we still know very little about population structure of alpine aquatic insects within smaller areas. In the High Tatra Mountains, there are more than 110 permanent lakes of glacial origin and many small ponds. In 2009, we promoted the study of genetic variability of aquatic insects of these biotopes starting with beetles Agabus bipustulatus and Agabus guttatus (Coleoptera: Dytiscidae). For haplotype analysis we used 350bp fragment of cytochrome b mtDNA. Among ca 450 samples, relatively small haplotype diversity was detected, with different distribution in both species. Most of lakes was characterized by single haplotype and majority of haplotypes were restricted to only one of sampled valleys. Consequently, the detailed analysis of microsatellites of both specie will follow. Results of analysis based on molecular methods can contribute considerably to the knowledge on the distributional trends, recolonization or identifying refugia of the species. Consequently they can help to preserve these unique aquatic environments and their biodiversity. Beside population study, the data on intraspecific variability will serve for development of determination technology of animal species using DNA. This contribution/publication is the result of project implementation (ITMS: 26240220049) supported by the R&D OP funded by the ERDF.

Keywords:*alpine lakes, aquatic insects, DNA, population-genetics, diving beetles*

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Ecology

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Is the univoltine papilionid, *Luehdorfia japonica* (Lepidoptera), declining by the climatic warming?

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The univoltine papilionid butterfly, Luehdorfia japonica (Lepidoptera), is endemic to the Honsyu Island in Japan. Adults appeared in and around 'Satoyama' coppices in spring, and larvae, which feed on leaves of Asarum herbs (Aristolochiaceae), pupate in early summer. The pupae successively undergo two kinds of diapause, summer and winter diapause terminated by autumnal short day and winter cold, respectively (Ishii and Hidaka, 1983). This species is ranked as an endangered species in Japan, but the extinction rate of population is higher in regions with warmer winters than those with colder ones (Ishii, 2010; Tanikawa and Ishii, 2010), suggesting that the recent climatic warming threatens this species. In this study, we reared eggs, larvae and pupae in the state of winter diapause under various temperature conditions in order to clarify the influence of hotter summer and warmer winter on the survival rate. As for the eggs and larvae, all individuals died at 30C, while about 90% of individuals survived at temperatures between 15 and 25C. Pupae were subjected to 9 temperature and photoperiodic conditions (Experiments 1-9) after the termination of summer diapause by a 2-month exposure to 20C-12L-12D. Adult emergence was observed in 6 experiments where pupae were subjected to 0 or 10C for 4 months before a transfer to 20C-16L-8D. In contrast pupae exposed to 20C-12L-12D for more than 2 months died at the pharate adult stage. The results demonstrate that this species is not able to survive summers as hot as 30C and winter as warm as 20C.

Keywords:*butterfly, Luehdorfia japonica, climatic warming, pupal diapause, survival rate*

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Ecology

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A trophic cascade effect of *Toxorhynchites rutilus* (Diptera: Culicidae) predation on aquatic bacteria

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Feeding by container-dwelling mosquito larvae may negatively affect microorganism richness or abundance. However, indirect effects of predators of mosquitoes on microorganisms are poorly studied. Larvae of the predator Toxorhynchites rutilus prey on larval Aedes triseriatus, which feed on bacteria. We postulated that a trophic-cascade arises from T. rutilus predation on A. triseriatus larvae, impacting bacterial abundance within water-filled containers. Experimental microcosms (450ml plastic cups holding 300ml of deionized water) received 100ml of oak leaf infusion (35mg/L, 9 days old) as a bacterial source and 100 first-instar A. triseriatus larvae. Treatments were: prey alone; prey with one T. rutilus larva; and prey with water-borne predatory cues, which consisted of water that had held one T. rutilus larva feeding on A. triseriatus larvae for 5 days. Controls were: infusion alone; or infusion plus predatory cues. We measured bacterial production via 3H leucine incorporation every 7 days14 days. As predicted, bacterial abundance was greater in the predator treatment than in others. Bacterial abundance did not differ between predator and predatory cues treatments. We hypothesized that predator and predatory cues treatments contain partially eaten prey, increasing bacteria abundance. We tested this in an experiment in which A. triseriatus were either crushed, crushed and removed, or subject to real predation. Controls received 100 first instar A. triseriatus larvae. Surprisingly, bacterial abundance was greater with real predation than in all other treatments. We suggest that feces (from either predator or prey) or reduced foraging induced by predator cues contributed to bacterial growth.

Keywords:*Aedes triseriatus, Toxorhynchites, mosquito container, insect ecology, medical entomology*

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Ecology

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Considering associate plant diversity to enhance biological control of *Dacryodes edulis* (G Don) in Gabon

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The monitoring of entomofauna among associated plants integrating Dacryodes edulis was carried out in Gabon in 2009 and 2010, using yellow traps and visual control. Two kinds of home gardens were described from the rural and urban areas where the studies occurred. Fifty one plant species were identified, with 47% of fruit trees, 23% of food crop plants and 29% of wild plants. Dacryodes edulis was the major (21%) of fruit trees in these home gardens. Our results showed that in home gardens dominated by fruit trees, the insect's abundance provided by yellow traps were higher (14897 insects) than in the home gardens with mixed fruit trees and herbaceous crops (13031 insects). Visual control allowed to record closely balanced abundance of insects, with 4901 insects on Dacryodes edulis from home gardens dominated by fruit trees, and 5188 insects on D. edulis of associate plant gardens. However, Shannon Index calculation shows no variation of insect's diversity recorded in both home gardens. The major pest caught by traps belong to Aphididae (20,2%), Cicadellidae(6,1%) and Psyllidae (22,8%), while those of visual control were Cecidomyiidae(8,9%), Crambidae(8,4%), and Stictococcidae (4,1%). In regards to these results, exploiting associated plants around D.edulis could avoid insect outspread and damage on this fruit tree. Further studies are to make in this way in order to enhance biological control of D.edulis in Gabon

Keywords:*Associate plants, Dacryodes edulis, Insects, Biological control, Gabon*

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Ecology

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Insects associated with *Jatropha curcas* in the Brazilian Cerrado

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Jatropha curcas L. (Euphorbiaceae) is an important alternative source for biodiesel production in Brazil and worldwide. Despite their hardiness, jatropha plants are often attacked by pests and diseases that can lead to severe losses. This study surveyed the insects associated with jatropha in the brazilian cerrados, and was conducted in an area of 1.3 ha, located at Embrapa Cerrados, Planaltina/DF (15 ° 35 '42.7' S, 47 ° 44 '14.8' W, 1039 m), with 3 to 4 year-old plants. Samples were collected biweekly for 6 months, between November/2011 and April/2012. Insects were collected on 10 randomly selected plants in the area, taking in account all above-ground plant parts. They were identified as to their functional group (chewing/sucking feeding habits, predators, parasitoids, detritivores and pollinators), order, family and morphospecies. In total, 905 insects were collected in 9 orders, 40 families and 109 species. The order Hemiptera was the most abundant (76%). Most of the insects were collected on the leaves. Individuals from all functional groups were collected, with emphasis on sucking insects (73%) and predators (15%). Pachycoris torridus (Scopoli) (Hemiptera: Scutelleridae) was the most abundant species (54%). The months with the highest number of insects collected were February (29%), March (26%) and January (22%). Knowledge of the main pests and their natural enemies brings key information for management strategies in this culture, as the area planted with jatropha increases in an impressive rate in Brazil.

Keywords:*Jatropha plants, Insects, diversity, Pachycoris torridus, biodiesel*

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The nocturnal egg parasitoid *Paratelenomus saccharalis*

Keiji Takasu
Kyushu University, Japan

Although host foraging behavior of many parasitoids has been intensively studied in the laboratory, it is unknown for most of the parasitoid what time of the day they are active in the field. In the present study, I discovered that the egg parasitoid Paratelenomus saccharalis are active from dusk to dawn. The host Megacopta punctatissima laid egg masses consisting of 10-40 eggs on buds and stems of the kudzu from 1100 to 1300. Paratelenomus saccharalis females appeared on or near host egg masses from 1600 to 1900. No female appeared on the host egg masses from 800 to 1500. Soon after arriving at host eggs, females rested near host egg masses without ovipositing. From dusk into night, they were parasitizing. It took 5-12h for a female to complete ovipositing in an average of 17 eggs before leaving a host. When a female parasitized a host egg mass, she left the hosts before 2400. However, when a female approached a host egg mass where another female had been parasitizing, the ovipositing female showed aggressive behavior toward the invading female. After finding another female, the resident spent more time patrolling and less time ovipositing. The invader attempted to sneak in to the host egg mass for oviposition unless she found another unoccupied egg mass nearby. When two females were on the same host egg mass, they remained the host egg mass and continued the war of attrition type competition for more than 12 hours or until next morning.

Keywords:*parasitoid, foraging behavior, aggressive behavior*

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Advantage of sex in aphids: A case study using *Acyrthosiphon pisum*

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A number of hypotheses have been proposed to explain the advantages of sexual reproduction over asexual reproduction. However, few attempts to test the hypotheses have been conducted because of difficulty establishing a good biological system. In the present study, we tried to test the advantage of sex over asexual reproduction by using aphids, for which we can experimentally switch sexual to asexual reproduction and vice versa. In northern Japan, the pea aphid, Acyrthosiphon pisum is obligatorily sexual in the wild, with the sexual generation in autumn. However, if the aphid is reared in the laboratory in a long photoperiod, they can continue to reproduce asexually. We had maintained 2 originally sexual clones asexually for 5 years before the experiments. Muller's ratchet hypothesis would expect the accumulation of deleterious mutations during the long period of enforced asexual reproduction. We successfully induced the sexual generation for the 2 clones by placing them in a short photoperiod, and crossed them to obtain fertilized eggs. We compared fitness components (viability and fecundity) between the offspring clones and the 2 parental clones at the same time. We observed many examples of genetically unfit offspring, i.e. failure of development in hatched larvae (fundatrices) and sterility of some adult fundatrices. As a result, our experiments indicated that all the offspring clones that once established had higher expected fecundity than did the parental clones. This result suggests that sexual reproduction led to appearance of healthy and fecund offspring by producing many unfit offspring, i.e. by purging deleterious mutations.

Keywords:*sexual reproduction, asexual reproduction, aphid*

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Ecology

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The flighty ability of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) tested using flight mill

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The study of its flight ability is essential to understanding the pattern of migration. Here, the flight ability of 1, 3, 5 and 7 day aged moths and the mated and unmated moths are tested using flight mill in laboratory. The results show that female moths of different age have different flight ability. The moths emerged after one-day show the worst flight ability, while those emerged after 3 days show the best flight ability, with a longest flight distance of 10.546 Km and flight duration of 11.613 h within 12 successive hours. The male moths emerged after 1, 3, 5 and 7 days show no difference with a longest flight distance of 13.191 Km and flight duration of 12.000 h. The mated and unmated male and female moths do not show difference in flight ability. After flight, the longevity and fecundity of the moth are reduced. However, the mated and unmated moths do not show different in longevity and fecundity after flight.

Keywords:*Plutella xylostella, flight ability, flight mill, longevity, fecundity*

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Ecology

P3

Annual fluctuation and food habits of *Hydrometra procera*

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Hydrometra procera is one of the semi-aquatic bugs found in paddy fields. I investigated the population fluctuation and food habits of the species to evaluate its potential as a biological control agent against Sogatella furcifera, Nilaparvata lugens, and Nephotettix cincticeps in a paddy field under sustainable cultivation. Through the present study, arthropods belonging to the following 7 taxa were sucked by the bug ;Onychiuridae gen. sp., S.furcifera, N.lugens, N.cincticeps, Chironomidae gen. sp., Brachycera gen. sp. And Theridiidae gen. sp. Population density of the bug in the paddy field showed two peaks a year, in late June and in September. The former peak tended to synchronize with a peak of S.furcifera nymphs , while the latter peak with N.lugens, N.cincticeps, and collembolans. Therefore, main food resource of the bug should be these insects in the paddy field. Ovarian development of the bug under field condition revealed that the bug could be uninvoltine since females possessing mature ovary appeared once a year, from June to Jurly. Rearing tests in laboratory at 22, 27 and 31℃ showed a developmental zero(T?) of 14.0℃ with a thermal constant(K) of 250℃-day from egg to adult for the bug, H. procera.

Keywords:*semi-aquatic bug, native natural enemy, collembolans*

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Ecology

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Predation,development and oviposition of *Hydrometra procera* (Heteroptera:Hydrometridae) under laboratory conditions

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Hydrometra procera is one of semiaquatic bugs in the paddy fields. We investigated the fecundity and predatory characteristics of this bug to evaluate the potential 23℃,25℃,30℃ and 35℃ under a 16L-8D photoperiod. The functional response of H. procera to prey density was saturation curve, and the maximum number of prey killed was 1.65 per day against WBPH dropped on the water surface. The averages of the total number of eggs laid per female were 10.5, 20.8,19.5 and 7.5 at 35℃,30℃,25℃ and 23℃, respectively. The total number of eggs laid were 100.8,186.2,223.2 and 64.1 at 23℃,25℃, 30℃ and 35℃, respectively. Survival rate of immature stages at 25℃ was significantly higher than at 23℃,30℃ and 35℃. The average of egg period were 5.9 days, 7.4 days and 8.7 days at 30℃,25℃ and 23℃,respectively. Egg period significantly decreased with increasing temperature. The five nymphal instars require 13.1 days at 25℃ to reach aduls . The development period of nymphs significantly decreased with increasing temperature.

Keywords:*semiapuatic bug, water measures, laboratory rearing, oviposition biological control agent, paddy fie*

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Ecology

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Quantitative genetics of the Desert Locust's larval growth: Rate and life-history strategy

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Schistocerca gregaria, the desert locust, which is distributed from north-west Africa to south-east Asia, is one of the most known and studied locust species. In order to prevent local human populations from the threat of pullulations, we need to know more about population dynamics of the desert locust, especially for the solitarious populations which are less studied than gregarious ones. Predicting the evolution of population dynamics needs a good knowledge of life-history traits that influence population demography. Growth is one of these traits, since it is directly linked to the onset of sexual maturity, then reproduction. Moreover, in order to investigate the potential of selection on individual growth, one needs to evaluate its heritability by disentangling genetic and additive variation in traits phenotype from non-genetic variance due to other (environmental) factors. Here we present a work done on a first generation lab population of S. gregaria sampled in the field. We recorded individual larval growth of 15 full-sib families, based on body weight and morphology in controlled conditions. We describe larval growth by analyzing growth rate as well as several key variables involved in life-history strategy (body weight and size at emergence, growth rate until adult emergence, maximum body weight, age at adult emergence, larval strategy). Thanks to the known relatedness structure of our sample, we also calculate the heritability of those traits and make predictions about the ability of S. gregaria populations to respond to selection on larval growth.

Keywords:*Desert locust, quantitative genetics, larval development, life-history strategies, heritability*

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Ecology

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Spatio-temporal dynamics of baculovirus infections in a major African crop pest, *Spodoptera exempta*

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African armyworm (Spodoptera exempta) is a major pest of cereals (maize, wheat, sorghum, rice) and pasture grasses in sub-Saharan Africa, especially in eastern Africa, where it is a major pest in 9 out of every 10 years. S. exempta is subject to a specific baculovirus infection, S.exempta nucleopolyhedrovirus (SpexNPV), which is being developed as a microbial pesticide for use in Tanzania. Here, we report on a 4-season study exploring the spatial and temporal dynamics of SpexNPV in natural African armyworm outbreak populations in Tanzania using PCR and qPCR to quantify both prevalnce and intensity of virus infections. We show that baculovirus levels increase through the season and vary in relation to both co-infection levels and population density in surprising ways.

Keywords:*baculovirus, NPV, African armyworm, Spodoptera exempta, outbreak pest, disease, biological control, microbial pesticide*

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Ecology

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Effects of latitude and type of surrounding agricultural land on abundance of the indigenous mirid bug *Pilophorus typicus*, a generalist predator of vegetable pests

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Introduction: The abundance of natural enemies on different types of agricultural lands can be affected by differences of latitude and the surrounding vegetation. Moreover, natural enemies in the surrounding vegetation can immigrate into agricultural lands as suppression factors of crop pest populations. Here, we report that effects of latitude and surrounding land use on the abundance of a generalist predator, Pilophorus typicus, on Kyushu Island in Japan. Materials and methods: Densities of P. typicus in 49 kudzu communities (31.25-33.19 °N) within 18 areas were determined in the summer and autumn of 2010. The following area rates of the surrounding agricultural lands were calculated in 11 spatial scales (95-3,000 m radiuses) with a vegetation map: 1) paddy-field, and 2) field, except paddy-field. Relationships of P. typicus density were analyzed with these field rates and latitudes of the kudzu communities. Results: P. typicus were collected in 29 kudzu communities (59.2 %, 31.25-33.15 °N). Densities of P. typicus decreased with increases in latitude of researched kudzu communities and the rate of surrounding paddy fields, and increased with an increase in the rate of surrounding fields, except paddy-fields. Conclusion: Conservation biological control programs using P. typicus in southern Kyushu are likely to be more effective than in northern Kyushu because of the abundance and immigration rate from kudzu communities. The results of the relationship between the abundance and agricultural lands can be used as references to evaluate the possible high immigration rate of P. typicus.

Keywords:*Pilophorus typicus*

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PS3M361

Ecology

P3

Spatial distribution and theoretic sampling sizes of oranges infested by *Bactrocera minax* in orchard

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The percentage of damaged fruit is a major parameter to estimate the extent of infestation caused by B. minax, and in certain degree, the spatial distribution of damaged fruits in orchard can reflect the oviposition action of B. minax. These data are the basis to establish an effective sampling technique. In this study, we investigated 118 citrus trees in an orchard in Jiang You, Sichuan province, China. Forty fruits were randomly investigated per tree, and the number of damaged fruits was recorded. The analysis of spatial distribution showed that the distribution of damaged fruits among different trees were aggregated distribution, which could be described as Neyman's distribution or Negative binomial distribution probability model. On the same tree, the damaged fruits randomly distributed, which could be described by Poisson probability model. According to the analysis of variance, the distribution of damaged fruits among different trees was significantly different (F = 1.7751, P = 0.000031), and no significant difference was detected in the distribution of the damaged fruits on the same tree (F = 0.4134, P = 0.7435). Based on the investigate data, taking the primary unit (tree) and second unit (fruit) into account, the best sample size was calculated using second order sampling model. The results indicate that in an

Keywords:*Bactrocera minax, spatial distribution pattern, sampling technique, citrus.*

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Ecology

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Genetically modified poplar as biofuel: Another opportunity for insect exploitation?

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Global energy demands have elevated biofuel research to an unprecedented level of prominence in the last decade. The plant cell wall itself poses perhaps the most significant barrier to the feasibility of biofuel technology. Genetic modification of biofuel crops to alter cell wall composition has proven to be a promising next step toward efficient conversion of cellulose to ethanol. But an important question arises that requires investigation by entomologists: Will insects over-exploit genetically modified biofuel crops? The purpose of this study was to investigate the effects of lignin modification in poplar on leaf chemistry and consequent insect resistance and insect herbivore prevalence. Leaf samples from several modified poplar genotypes were chemically analyzed and assessed for damage by insect herbivores. Surveys were conducted to determine prevalence of insect herbivores and their natural enemies. No significant differences were found in leaf chemistry, insect resistance or prevalence, suggesting genetically modified biofuel poplar for altered lignin composition may in fact maintain resistance to insect herbivores.

Keywords:*biofuels, poplar, genetically modified, resistance*

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Ecology

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Structure determination of the elicitors in the *Sogatella furcifera*(HORVATH) which make japonica rice plant (Oryza sativa L.) varieties induce ovicidal substance against the egg of *S. furcifera*

Jeong-Oh Yang[1], Naoya Nakayama[2], Chul-Sa Kim[3]

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Some japonica rice plant (Oryza sativa L.) varieties showed a resistance that is characterized by formation of watery lesions and production of ovicidal active compound, benzyl benzoate (B.B.) when the whitebacked planthopper lays eggs in the varieties. This B.B. results in a high mortality to eggs of S. furcifera and reduces to egg hatchability. We, therefore, have tried to elucidate mechanism for this resistant and to isolate and identify the elicitor(s) in S. furcifera. B.B was produced with the formation of watery lesions, only when 80% MeOH extract (20females of S. furcifera equivalent) was applied on to the surface of the rice plant (ver. Natsuhikari) after the plant was hurt by the sewing needless. This result clearly shows that the elicitor(s) in the S. furcifera makes japonica rice plant induce ovicidal substance against the egg of S. furcifera. The elicitor(s) were eluted in the 100% MeOH and 100% EtOH fractions (frs) on a reversed phased ODS open column. The both frs induced B.B. at 43.95 μg/g of fresh rice plant (frp) and 31.04 μg/g frp, respectively. The further separation of the active 100% MeOH fr by using a reverse phased HPLC column leads to isolate five active compounds and the structures of each compound were determined by using NMR, LC-MS and GC-MS spectra as 1, 2-dilinoleoyl phosphatidyethanolamine (22.4 μg/ 20 females), 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine(3.32 μg/ 20 females), 1-palmitoyl-2-oleoyl-X-glycero-3-phosphoethanolamine (7.96 μg/ 20 females) and 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine (5.12 μg/ 20 females), respectively.

Keywords:*Sogatella furcifera, benzyl benzoate, 1, 2-dilinoleoyl phosphatidycholine, 1, 2-dilinoleoyl phosphatidyethanolamine, 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, 1-palmitoyl-2-oleoyl-X-glycero-3-phosphoethanolamine, 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine, ovicidal compound, elicitor,*

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Ecology

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Comparison of life-history traits of two cryptic species in *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)

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Brontispa longissima is a serious pest of the coconut Cocos nucifera supposedly originated from Papua New Guinea and Indonesia. It has recently invaded into Southeast and East Asia and its outbreaks have been reported in there. Our mitochondrial DNA analysis revealed that there are two cryptic species in B. longissima: one is distributed over a wide area including Asia and the Pacific region (herein referred to as the Asian clade) and the other in a limited area in the Pacific region (herein referred to as the Pacific clade). The distribution of Asian clade coincided with the area of recent invasions and outbreaks of this species, suggesting that only the Asian clade has become a serious invasive pest. To determine whether the Asian clade is more invasive than the Pacific clade, we compared life-history traits of the two clades on C. nucifera. Insects of the Asian clade showed larger net reproduction rates (R0), intrinsic rates of natural increase (r), and mean length of a generation (T) than Pacific clade (R0: 130.0 and 94.0, r: 0.084 and 0.083, and T: 57.7 and 54.7 in the Asian and the Pacific clade, respectively). Even there was small difference in r (0.001), the simulated population growth indicated that the number of the insects of the Asian clade would be twice and three times as many as that of the Pacific clade after five and ten generations, respectively. Rapid population growth was considered to be a factor of wide distribution and outbreaks of the Asian clade.

Keywords:*Cocos nucifera, intrinsic rate of natural increase, invasive species, longevity, net reproductive rate*

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Ecology

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Does seasonal change of chemicals in the primary host plant, *H. syriacus*, stimulate the host shift by the cotton aphid (*Aphis gossypii*) from the primary host plant to the secondary host plant?

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The cotton aphid (Aphis gossypii) is a polyphagous serious pest in the world and gives many vegetables and crops damage. This species has a complex life cycle. In early spring, the cotton aphid hatched on a primary host plant, Hibiscus syriacus, has spent on it through three or four generations and then shifts the secondary host plant, for example, cucumber, egg plant and so on in early summer. In autumn, the male and female re-come back to H. syriacus and the female lays egg on it after mating. Why must the cotton aphid shift the host plant? Why cannot the cotton aphid live on the primary host plant through four seasons? We try to elucidate the reason in the view of the seasonal change of quality and quantity of chemicals in primary host plant, H. syriacus. As a result, we found that concentration of uridine became to be the maximum before a few days at the time of maximal nymph's population and that concentration of fumaric acid increased along with increase in number of alatae. When several combinations of the chemicals in the leaves of H. syriacus were artificially given to the cotton aphid through palafilm membrane with an artificial diet, the combination including fumaric acid with the maximum concentration tended to stimulate appearance of alata in next generation.

Keywords:*cotton aphid, uridine, fumaric acid, alata*

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Ecology

P3

V-ATPase is involved in silkworm defense response against *Bombyx mori* nucleopolyhedrovirus

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Silkworms are usually susceptible to the infection of Bombyx mori (B. mori) nucleopolyhedrovirus (BmNPV), which can cause significant economic loss. However, some silkworm strains are identified to be highly resistant to BmNPV. To explore the silkworm genes involved in this resistance, in present study we performed comparative real-time PCR, ATPase assay, over-expression and sub-cellular localization experiments. We found that when inoculated with BmNPV both the expression and activity of V-ATPase were significantly up-regulated in the midgut column cells (not the goblet cells) of BmNPV-resistant strains (NB and BC8), the main sites for the first step of BmNPV invasion, but not in those of a BmNPV-susceptible strain 306. Furthermore, this up-regulation mainly took place during the first 24 hours post inoculation (hpi), the essential period required for establishment of virus infection, and then was down-regulated to normal levels. Amazingly, transient over-expression of V-ATPase c subunit in BmNPV-infected silkworm cells could significantly inhibit BmNPV proliferation. To our knowledge this is the first report demonstrating clearly that V-ATPase is indeed involved in the defense response against BmNPV. Our data further suggests that prompt and potent regulation of V-ATPase may be essential for execution of this response, which may enable fast acidification of endosomes and/or lysosomes to render them competent for degradation of invading viruses.

Keywords:*Bombyx mori, B. mori nucleopolyhedrovirus, V-ATPase, defense response*

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Ecology

P3

Descriptions and comparison of antennal morphology and sensilla ultrastructure of three *Tomicus* species (Coleoptera: Curculionidae, Scolytinae)

Zhen Zhang[1], Pingyan Wang[2], Xiangbo Kong[3], Hongbin Wang[4], Sufang Zhang[5]

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The antennal morphology and sensilla ultrastructure of Tomicus yunnanensis, T. minor, and T. brevipilosus were studied by scanning (SEM) and transmission electron microscopy (TEM). The three species had similar morphology and sensilla type, but sensilla on antennal club segments 2, 3, and 4 in T. minor were much sparser than in T. yunnanensis or T. brevipilosus. Eight sensilla types were recorded: sensilla trichodea types 1 and 2 were located apically and on the lateral surface of the club and were innervated by five and eight dendrites, respectively; sensilla chaetica types 1 and 2 had no dendrites in the sensilla lymph lumen; sensilla basiconica, top-protuberated sensilla basiconica, and fluted cones occurred primarily on the club; and Bohm bristles occurred on scape segment 1. Sensilla basiconica were the most abundant and were innervated by 10-14 dendritic branches. Top-protuberated sensilla basiconica and fluted cones were innervated by one and five dendrites, respectively. The potential functions of these sensilla are discussed with respect to morphology and ultrastructure. We compared the sizes and numbers of each sensilla type between sexes, within species, and between two development phases. There were no statistical differences between sexes. The lengths and numbers of sensilla basiconica and sensilla trichodea type 2 differed significantly among the three species.

Keywords:*Tomicus, Sensilla, Morphology, Ultrastructure, Chemoreceptors, Mechanoreceptors*

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Ecology

P3

Predicting the risk of extinction for moths and butterflies by using shared ecological features of species and its larval food plants

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Recent studies predict that endangered species of butterflies are characterized by narrow niche breadth, poor ability to colonize suitable habitats and short flight period. Majority of works are focusing on butterflies and testing the effect of ecological features of species on their extinction risk. We are convinced that the inconsistent results of previous works are caused by neglecting ecological characteristics of their larval food plants. Butterflies and geometrid moths of the Czech and Slovak Republics (Central Europe) were chosen as a studied group of herbivores. Index of population distribution (proportion of occupied faunal squares among old and new records) was used as dependent variable for butterflies, whereas conservation status was used for geometrid moths. Effect of ecological characteristics of species and larval food plants were fitted by using the method of generalized least squares (GLS). Our analysis predicts that threatened butterflies and geometrid moths are characterized as thermophilous and heliophilous species preferring steppe habitats, with Mediterranean type of distribution. The best fitted characteristics of larval food plants for predicting the degree of extinction risk were (i) plant species frequency and period of a host plant flowering for the moths; (ii) Raunkiaer plant life-forms, species frequency in grid maps and nitrogen preference of plants for butterflies. Ecological characteristics of the moths explain 14% of the total variability, however, 4% of the variability are explained by characteristics of food plants. On the other hand, ecological features of the butterflies explain 54% of variability and 16% are explained by characteristics of food plants.

Keywords:*Rhopalocera, Geometridae, ecological features, risk prediction, conservation*

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PS3M369

Ecology

P3

The influence of severe drought on the resistance of *Pinus yunnanensis* to a bark-beetle-associated fungus

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Researches on tree response to pest attacks under drought and heat stress are crucial for predicting effect of future climate change on forest pests and tree health. We studied the influence of severe water stress on the resistance of Pinus yunnanensis to inoculation with Leptographium yunnanense, a pathogenic fungus associated with the aggressive bark beetle, Tomicus yunnanensis. Experiments were carried out during May to September 2010 and March to April in 2012 in two plots located at the top and at the foot of a hill in Xiaojiu, China. Following isolated and mass fungal inoculations, we observed the reaction zone length, fungal growth in the phloem, and the occlusion, blue-staining of the sapwood. Measurements of soil and needle water contents and predawn needle water potentials confirmed severe drought was occurred during the experiment time, especially at hilltop. Measures of fungal infection of phloem and sapwood in June were more serious than normal year. Reaction zone length and fungal extension negatively correlated with ψ. The results suggest that severe water stress under high temperature has a negative impact to tree resistance to fungal pathogens associated with Tomicus yunnanensis.

Keywords:*severe drought, isolated inoculations, Leptographium yunnanense, mass inoculations*

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Ecology
P3

Various groups of invertebrates ? various responses for the proposal of "biodiversity-friendly management" of submontane meadows. Is there any rational compromise?

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The aim of this study was to evaluate impact of agricultural management and the landscape structure on insects of semi-natural grasslands. The investigated sites were flower-rich Arrhenatherion meadows with variable schemes of mowing in the submontane region of the Hruby Jesenik Mts. (Czech Republic). Insects were sampled using Moericke´s yellow traps. The response from selected groups of insects (mostly nectarivorous and phytophagous species) of changing vegetation characteristics and the landscape structure had been investigated during two vegetation seasons (2009-2010). Data were analysed using multivariate ordination technics (CCA, GLM), intensity of mowing, number of flowering plants, distance from grass balks and forest edges being independent variables. According to the results of analyses, each group of insects responses particularly different on the changing environmental conditions reflecting the agricultural measure. Nevertheless, the main role played factors of seasonality and the position of both interaction components (i.e. grass balks, forest edges). Contrary to other recent studies, immediate impact of the mowing was detectable up to the particular species (e.g. Hesperia comma, Lasiommata maera) or taxa (Bombus spp.), but not in the whole community level. On the base of this study, we can summarize: (i) the majority of insects´ abundance is localized near the margins of meadows, (ii) the highest abundances of invertebrates were collected in the main vegetation season (June, July). Therefore, (iii) the most destructive management measure in the long-term scale is the extensive mowing in the main season, covering the edge parts of the meadows.

Keywords:*insects, biodiversity,conservation, grasslands, mowing, landscape structure*

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Ecology

P3

Use of climate-based model for better understanding spatial distribution of Encyrtidae (Hymenoptera: Chalcidoidea) in North-Western Asia

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Successful use of Encyrtids as pest biocontrol agents is one of the important challenges today. In spite of this, available data of the ecology and distribution of Encyrtids are rather scarce and imperfect. There is no data to what environmental factors limit or promote (if any) the distribution of the Encyrtid species. In the present study, we collect all available distributional data for Encyrtid species from Iran, Turkey and Transcaucasian countries. Based on the collected data we produce climate based species distribution models and extrapolate them to whole northern temperate zones. Based on the received results, we have identified most species reach regions within the study area (a); for each species we have predicted the suitable habitats for potential use in future introduction (b). Our results suggest that additional investigations are needed to assess the faunal diversity in the studied region.

Keywords:*Encyrtids, Georgia, Armenia, Azerbaijan, Turkey, Iran*

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PS3M372

Ecology

P3

Life history of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on three cotton *Gossypium hirsutum* L. varieties

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Cotton armyworm Spodoptera litura when reared on CB9, CB10 and SR05 cotton varieties showed that preoviposition and incubation periods of the moths did not differ significantly but fecundity and hatching rate were significantly different. The highest fecundity (1656.8 ± 270.6) and hatching rate (76.3 ± 16.2%) were found when the moths were provided with SR05 and CB10, respectively. Cotton varieties had significant effects on larval, prepupal and pupal periods. The highest larval (33.4 ± 2.7 day), prepupal (3.8 ± 0.4 day) and pupal (17.4 ± 1.3 day) periods were observed on CB10, CB9 and CB9, respectively. The highest pupation (92.0 ± 10.9%) and adult eclosion (81.0 ± 10.8%) rate were found on CB10 and SR05, respectively. The mating behaviour of the moths differed according to cotton variety; the longest mating duration (123.5 ± 15.3 minute) was found on SR05, compared to CB9 (102.6 ± 15.0 minute) and CB10 (113.3 ± 21.0 minute). The moths showed significantly higher mating success when they were reared on CB10. Cotton varieties did not show significant effect on the remating success of males and females. The longevity of adults varied significantly and the highest longevity of male (10.5 ± 0.9 day) and female (8.9 ± 0.8 day) moths were found when they were fed SR05. Overall, S. litura fitness appears to be highest on cotton variety SR05, suggesting that this variety is least suitable of those tested here for use in areas where S. litura is a major pest.

Keywords:*Cotton variety, life history, Spodoptera litura*

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Ecology

P3

A climatic variables-based prediction on the distribution of an alien invasive hornet *Vespa velutina nigrithorax* in Korean Peninsula

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Vespa velutina nigrithorax is a hornet that inhabits subtropics such as southern China, Indonesia and Taiwan. Meantime, this alien invasive hornet was detected in Busan city, Korea in 2003. Furthermore, this species has been increasing continuously in the Korean Peninsula, especially in its southland that has been being a subtropical region with climatic changes. In 2011 it was found that this species spread to Daegu city to the north and to Jiri Mountain to the west, and in Busan city and the surrounding area it has been dominant over other Vespa species. Hereat, a prediction was made about the spread and distribution of the species by the application of 19 climatic variables to the maximum entropy algorithm, whereof results were analyzed by use of the Maxent species distribution prediction program. In result, this species was predicted to spread throughout the nation after proceeding southwestward and then northward. In particular, the southland is predicted to meet optimal climatic conditions for the inhabitation of this species within 2050 and the whole territory, excluding some parts of Gangwon Province and Gyeonggi Province, is predicted to do so within 2080. Their spread, of course, will exceed the foregoing climatological prediction, but it will take them the estimated time to be completely naturalized or dominant. The data, derived from this study, are expected to bestead the preparation of countermeasures for ecological disturbance, economic impacts and public health problems.

Keywords: *Vespa velutina nigrithorax, alien invasive hornet, spread, climate change, prediction*

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Ecology

P3

Damage type of glasswort by *Scrobipalpa salinella* (Zeller) (Lepidoptera: Gelechiidae) and Occurrence by Region

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Damages of Scrobipalpa salinella by farming management types were divided by three types on glasswort. Type 1 is that growers not clean up debris in the farming period. Type 2 is that growers left dried glasswort not harvested in the previous year. Type 3 is that grower left the field mixed with around weed. Occurrence of Scrobipalpa salinella (Zeller) was investigated on glasswort in Shinan and Suncheon, Jeonnam and Kimjae, Jeonbuk in 2011. The occurrence of S. salinella observed from four times (in Shinan and Kimjae) to five times (in Suncheon) in a year. Adult stage of S. salinella in Shinan areas (Jeungdo, Imja island) occurred from mid-April to late September. The peak times of 1st, 2nd, 3rd and 4th generation adults of S. salinella were observed on early May, mid-June, early August and early September, respectively. In Kimjae area, the peak times of 1st, 2nd, 3rd and 4th generation adults of S. salinella were observed on early May, mid-June, late July and mid-September, respectively. In Suncheon area, the peak times of 1st, 2nd, 3rd, 4th and 5th generation adults of S. salinella were observed on early May, mid-June, early August, early September and early October, respectively.

Keywords:*Glasswort, Scrobipalpa salinella, Damage, Density, Occurrence*

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Ecology

P3

Damage analysis and control method of *Laodelphax striatellus* (Fallen) (Homoptera: Delphacidae) on rice

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This study was carried out to know the damage aspects of rice and to develop control method of small brown planthopper (Laodelphax striatellus). The damage aspects of rice by L. striatellus at different population densities (1, 2, 3, 4/plant at seedling, 10, 20, 30, 40/plant at tillering stage) and different growth stages (seedling, tillering) were studied in rice field. The yield loss was more higher seedling stage than tillering stage of rice. The economic threshold level of L. striatellus which caused 50% loss of yield were estimated to be approximately 2 for the seedling stage and 12 for the tillering stage per plant, respectively. In the experiment, the linear relationships between density and decrease of yield were as following; seedling stage and tillering stage was Y= 4.0097X-1.6874 (R2=0.9272) and Y= 0.5281X-1.2034 (R2=0.9852), respectively. The days of control effect which caused over 80% were estimated to be approximately 20 days for seed box treatment and 40 days in seed-dressing plot of Imidacloprid compared with that of untreated plot, respectively.

Keywords:*Rice, Laodelphax striatellus, Damage, Analysis, Control method*

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PS3M376

Ecology

P3

Introduction of *Tanysphyrus* (Tanysphyroides) major Roelofs (Coleoptera: Curculionidae), a possible candidate agent for the biological control of *Monochoria vaginalis* var. plantaginea (Roxb.) Solms in

Jinyoung Park[1], Jong Kyun Park[2]

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Monochoria vaginalis var. plantaginea (Pontederiaceae) is one of the most problematic weed in the rice field in Korea. Tanysphyrus (Tanysphyroides) major was selected as a potential biological control agent for M. vaginalis var. plantaginea. This species has a single generation per year, over-wintering as an adult stage. The emergence of adults starts in later June and last until September. Host specificity test showed that finally selected this species was suitable candidates for the biological control of M. vaginalis var. plantaginea since it showed negative host specificity against major 60 test crops. Also, the thirtythree plants of the M. vaginalis var. plantaginea were planted for the T. (T.) major releasing experiment. Every experimental plots planted with M. vaginalis var. plantaginea were treated with the adults of regulated 35, 50 and 100 pairs. Control effect to M. vaginalis var. plantaginea by the adults density of T. (T.) major were investigates. When the 25, 50 and 100 adult pairs treated in the middle stages of M. vaginalis, required period for effective biological control were 7, 6 and 4 weeks, respectively. Also, M. vaginalis var. plantaginea injured by the T. (T.) major were not regrowth and fruits. T. (T.) major adults when input 25 and 50 pairs, necessary time were required 14 weeks for biological control of later stages M. vaginalis var. plantaginea, and seed investigated number of 13,231 and 10,157, respectively. And case of 100 pairs was 11 weeks, and seed investigated number of 4,469. Seed weight was 1.63, 1.24 and 0.48g, respectively.

Keywords:*Tanysphyrus (Tanysphyroides) major, Monochoria vaginalis var. plantaginea, biological control agent*

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Ecology

P3

Genetic diversity of the long-horn beetle, *Callipogon relictus* (Coleoptera: Cerambycidae) in North Korean and Chinese populations

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The long-horn beetle, Callipogon relictus Semenov, is distributed in Northeast Asia including South and North Korea, China, and Russia, but the populations have drastically decreased for last a few decades because of habitat destruction. So this species was listed as an "endangered wildlife species" in South Korea as in other countries. In order to establish a conservation strategy and select introduced populations, a preliminary investigation on the genetic diversity of North Korean and Chinese populations was performed using mitochondrial Cytochrome c oxidase I (COI) (470bp) and COII (718bp). A total of 17 individuals of C. relictus was collected from 3 locations in North Korea (2 locations) and China (1 location) and analyzed with respect to variability within and differentiation among populations. Partial COI and COII gene of C. relictus showed low genetic variation (COI:0.0017?0.0146, COII:0.0024-0.0039) within and differentiation (COI:0.0046-0.0124, COII:0.0026-0.0038) among populations, respectively.

Keywords:*Long-horn beetle, Cerambycidae, COI, COII, conservation*

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Ecology

P3

The Influence of mine leachate on community structure of benthic macroinvertebrates

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This study was conducted for determining the influence of the mine leachate on the community structure of benthic macroinvertebrates from Uiseong Okdong Mine, which located in Uiseong-gun. On August 30, 2011 an examination was made of the benthic macroinvertebrates and water quality from three sites on the experimental water system by the mine leachate from Uiseong Okdong Mine and those from 2 sites on the control water system located near the experimental water system. The identified species and individuals number were 9 species belonged to 8 families, 6 orders, 4 classes, 3 phyla and 122.1 inds./m2 in experimental area and control areas were 14 species belonged to 11 families, 6 orders, 4 classes, 3 phyla and 1546.5 inds./m2. From the mine leachate analysis, a high concentration of Cu, Zn, Cd, and Pb was detected, and their levels of pH was measured to be low and their Electric conductivity was high. Spearman's rank correlation coefficient between species number and physico-chemical factors (pH, electric conductivity, Cu, Zn, Cd, Pb) had significant correlations.

Keywords:*mine leachate, benthic macroinvertebrates, water quality*

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Ecology

P3

Dredging effects of check dam on benthic macroinvertebrate communities of mountain stream

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This study was conducted for determining the dredging effects of check dam on benthic macroinvertebrate communities. The check dams were surveyed before and after dredging from mountain streams of Yecheon-gun, Yeongju-si and Bonghwa-gun. Sampling was taken at upper and down from 3 experimental sites and 2 control sites during May to August 2011. The identified species before dredging were 38 belonged to 23 families, 10 orders, 5 classes, 4 phyla and 1575.3 inds./m2, but after dredging it has decreased to 21 species belonged to 16 families, 7 orders, 4 classes, 3 phyla and 1055.6 inds./m2. The microhabitat damage of benthic macroinvertebrates occurred at experimental sites after dredging of check dams led to reduction of an average of 38% species. In general, diversity indeces and richness indeces decreased after dredging; however, dominance indeces increased. In the upper stream of experimental sites, the microhabitat damaged and became pool due to dredging. Also, the river bed became simply due to the sedimentary matter being finer than sand and silt. As a result, composition of functional feeding groups and functional habit groups were relatively simpler at upper stream and the degree of community differences was greater between upper and down stream. Community stability by survey, an environment of water system in experimental area started to be somewhat destroyed, after dreding, it is shown that both species in I area which have great ability of resistance and recovery and species in III area which live in relatively stable water system decreased a little.

Keywords:*check dam, benthic macroinvertebrates, dredging*

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Ecology

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The influence of dam construction on benthic macroinvertebrate communities of down stream

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This study was conducted for determining the influence of dam construction on benthic macroinvertebrate communities of donwnstream. Sampling was taken at 5 experimental sites in downstream areas of Gunwi dam(1 site), Yecheon pumped storage power plant's upper dam(2 sites) and lower dam(2 sites) in Gyeongsangbuk-do of Korea. The benthic macroinvertebrate communities of these dams were surveyed during March 2008 to October 2011. The habitat damage of benthic macroinvertebrates occurred at the experimental sites during dam construction activities and led to a reduction of the number of species and individuals. In general, species diversity indices and richness indices decreased every year; however, dominance indices increased at experimental sites. Composition of filtering collectors increased each year and Hydropsychidae spp. which have greater ability of resistance and resilience and belong to filtering-collectors increased each year. Biological water quality evaluation using the ESB and BIBI was found that decline annually. Spearman's rank correlation coefficient between biological attributes and water quality parameters had significant correlations, particularly in SS and TP. It was found that SS and TP played an important role in structuring benthic macroinvertebrate communities. In conclusion, this study found that disturbances caused by dam construction activities had negative effects on benthic macroinvertebrate communities thus leading to suspended soils occurrence at stream ecosystem.

Keywords:*dam construction, benthic macroinvertebrates, community analysis*

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Ecology
P3

Edge effect of ground beetles in secondary forests

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We examined the edge effect on ground beetle assemblage in the edge of secondary forests in Hwaseong-si, Gyeonggi-do. Nine secondary forests were selected from highly fragmented urban landscape, and ground beetles collected by pitfall traps during 14 June to 9 November, 2011. A total of Eighty-one pitfall traps were installed along forest interior (+20m)—forest edge (0m)—forest exterior (-20m) gradients in each secondary forest. The species richness was rather higher in forest exteriors (ANOVA, with Tukey's test, F2, 77=0.03, P=0.9670), while abundance was significantly higher in forest edges and forest interiors (F2, 78=6.19, P=0.0032). Non-multivariate dimensional scaling showed a significant difference between forest exteriors and forest edges/forest interiors (Stress=0.13), suggesting that all three habitats had a distinct ground beetle assemblage (ANOSIM, Global R=0.314; P=0.001). By two-way indicator species analysis, Dolichus halensis halensis was an indicator species for the habitat of forest exteriors, while Synuchus cycloderus was an indicator species for forest edge and forest interiors. Because secondary forests in our study had a few forest species, forest edges in our study sites did not showed high species richness possibly. Nevertheless, conservation and management of the edge of secondary forests are important, because forest edges generally provide habitats for overwintering and reproduction of many predaceous arthropods, especially ground beetles.

Keywords:*edge effect, conservation, biodiversity, ground beetles, secondary forest*

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Ecology

P3

Community structure and functional habit groups of benthic macroinvertebrate in nature stream and urban stream

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This study was conducted for searching the fauna, community structure and community indices of the benthic macroinvertebrate on Wicheon Stream(Nature Stream) and Geumho River(Urban Stream). The samples were collected 2 sites of each stream during May to October, 2011. In Nature Stream, the identified species were 62 species, 32 families, 12 orders, 5 classes, 4 phyla. Insecta were collected with Ephemeroptera 20 species(32.26%), Trichoptera 14 species(22.58%), Odonata 10 species(16.13%), Diptera 9 species(14.52%), Hemiptera, Megaloptera 1 species(1.61%), and the non-insecta were collected with Basommatophora 2 species(3.23%), Tricladida, Mesogastropoda, Archioligochaeta, Rhynchobdellida, Arhynchobdellidae 1 species(1.61%) respectively. And Urban Stream, the identified species were 36 species, 21 families, 10 orders, 6 classes, 3 phyla. insecta were collected with Ephemeroptera 15 species(41.67%), Trichoptera 6 species(16.67%), Diptera 5 species(13.89%), Coleoptera 1 species(2.78%), and the non-insecta were collected with Mesogastropoda, Rhynchobdellida, Arhynchobdellidae 2 species(5.56%), Veneroida, Archioligochaeta, Isopoda 1 species(2.78%) respectively. In the functional habit group, the Ephemeroptera and Trichoptera which belong to CB(Climber), SP(Sprawler) Nature Stream and in order of SW (Swimmer), BU(Burrower) Urban Stream.

Keywords:*benthic macroinvertebrate, nature stream, urban stream*

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Ecology

P3

Temperature effects on development on overwintering *Sericinus montela* Gray (Lepidoptera: Papilionidae) pupa

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The dragon swallowtail, Sericinus montela Gray (Lepidoptera: Papilionidae), is an important endemic species of Korea because the risk of extinction and habitat loss are increased by human activity including agriculture (cropland, pasture, irrigation, and pesticides), and urbanization. S. montela distributed from central China to the Amur and Ussuri regions. S. montela larvae feed on Aristolochia contorta (Aristolochiaceae). The lower developmental threshold and thermal constants of S. montela overwintering pupae were studied at four different constant temperatures (15, 20, 25 and 30°C) with photoperiod 10:14 (L:D). The lower developmental thresholds of female, male and both sexes combined were 12.38, 12.16, and 12.37°C, respectively. The developmental time of overwintering pupae decreased with increasing temperature between 15 and 30?C. Thermal constants of female, male and both sexes combined were 220.26, 192.31, and 200.18DD, respectively. The relationship between the developmental rate and temperature was fitted by linear and five nonlinear developmental rate models (Logan 6, Lactin 1, 2 and Briere 1, 2). The adult emergence variation of overwintering pupae was described by the two-parameter Weibull distribution model. The temperature-dependent adult emergence model of S. montela developed in this study could be used to develop the full-cycle phenology model of S. montela and applied to understand the effects of climate change on the emergence distribution of overwintering pupae.

Keywords:*Sericinus montela, temperature, linear and nonlinear developmental rate model, Weibull distribution*

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Ecology

P3

Cold tolerance of overwintering eggs of *Lycorma delicatula* (White) (Hemiptera : Fulgoridae)

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Lycorma delicatula is an important invasive pest in Korea. Overwintering is a key element of its life history, having large proportion of the life cycle (e.g. 6~7 months/year). In this study, we investigated the seasonal change of cold tolerance on field collected eggs of L. delicatula. Egg collection was made five times, approximately in monthly intervals, in Cheonan from 22 November, 2011 to 1 April, 2012. To examine the effect of cold acclimation, eggs were chilled for 1 week at 1 ℃. Control group was not chilled. For both groups, eggs were treated by the combinations of different temperatures (-5℃, -10℃, -15℃ and -20℃) and exposure time (1hr, 6hr, 12hr, 24hr, 3days, 5days, 7days and 10 days) at each sampling date. Cold tolerance ability showed the temporal changes during the winter season. Egg hatching rate was increased by cold acclimation. Critical temperature appears around -20 ℃ because all eggs treated at -20℃ failed to hatch. For field validation, we collected eggs from six different locations on 22~25 February in 2011 and collected eggs were placed at 25℃. There was significant correlation (r=0.975) between egg hatching rate and the mean low temperature from January 1 to February 28.

Keywords:*Lycorma delicatula, invasive pest, cold tolerance, overwintering*

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Ecology

P3

Community analysis based on functional feeding groups of benthic macro-invertebrates in Mankyeonggang(R.)

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This study is executed to obtain basic data to manage the water quality data of the Mankyeonggang(R.) systematically and to evaluate the biological effect of water quality of the tributary stream to the main course of the Mankyeonggang (R.). Collecting was executed at 7 sites of the main stream (2 upper streams, 4 mid streams, and 1 down stream) and 4 sites of tributary stream (Soyang, Jeonju, Iksan, and Top stream) from May to September 2009. It was also executed for quantitative and qualitative analysis of species diversity of benthic macro-invertebrates of community structure, local ecological characteristics and climate change using the collection, which was collected at the whole investigation sites. A total 114 species of benthic macro invertebrates in 55 families, 19 orders, 7 classes, and 4 phyla were collected during the field surveys. The result of EPT index value showed 44.7%, 51 species of EPT taxa, and it means that the stream ecosystem of Mankyeonggang (R.) is comparatively clear and healthy. Analysing FFG, predator was 37%, which was the highest. Then gathering-collector was 27%, filtering-collector was 15%, scraper was 11%, shredder was 6% , and plant-piercer was 4%. The cluster analysis of aquatic insects lists of species were executed using the similarity index. Therefore, they were divided into 2 groups (A and B), which were A-1(S1), A-2(S2~S7) and B(S8~11).

Keywords:*Functional Feeding Groups, Similarity Index, Cluster Analysis, EPT Index.*

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Ecology

P3

Insects diversity by habitat types in Middle Inland of DMZ, Korea

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This study was intended to understand the insects diversity and forest in DMZ(de-militalized zone) , and provide data and information required to preserve living creatures and geological management, and basic information for the management and preservation policies of insect diversity in DMZ. This study has surveyed insect diversity by habitat types in Middle Inland of DMZ, KOREA from 15 to 19 September, 2009. Totally there were 128 species belonging to 53 families of 10 orders and among them, 7 endemic species, 3 management of exotic species and 25 designated species were showed. The highest was 26 species of Hemiptera (20.31%) and then next are 22 species of Coleoptera (17.19%) and 20 species of Hymenoptera (15.63%) respectively. Dominant species are Ceracris nigricornislaeta (Bolivar) (0.152%) in S1, Menida violacea Motschulsky (0.218%) in S2, Stomorhina obsoleta (Wiedemann)(0.171) in S3, Ducetia japonica (Thunberg)(0.212%) in S4, Oedaleus infernalis Saussure (0.178%) in S5, Sepedon aenescens Wiedemann (0.268%) in S6, Adelphocoris triannulatus (Stal)(0.257) in S7 and Ricania taeniata Stal (0.150) in S8 site. The diversity (H') and richness (RI) of insects at the survey areas as S2 (H'=3.461, RI=9.64), S3 (H'=3.457, RI=9.18) and S1 (H'=3.447, RI=8.88) were higher than in the others and the lowest in S8 (H'=2.790, RI=5.341). But the highest species evenness index (EI) occurred in S8(0.985).

Keywords:*DMZ, INSECT DIVERSITY, VEGETATION, MANAGEMENT, PRESERVATION*

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Ecology

P3

Understanding of diversity and remarkable insects in urban green space - Botanic garden of Wonkwang University, South Korea

Soeng-Joon Park[1], Min-Ho Lee[2], Jae-Won Kim[3], Eui-Jeong Hong[4], Yong-Lak Jeon[5], Ju-Chang Yoon[6], Jin-Yung Kim[7], Byung-Jin Kim[8]

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This study was intended to see the insect diversity in Urban Green Space, special habitats in university campus, and provide data and information required to preserve living creatures and geological management, and basic information for the management and preservation policies. This study has surveyed insect diversity by habitat types in the botanic garden of Wonkwang university, Iksan, Jeonbuk, South Korea from September, 2010 to September, 2011. During the research period, a total of 209 species were observed, 78 families of 11 orders. Among them, 1 was a management of exotic species and 32 was designated species. The highest was 76 species of Coleoptera (36.36%) and next was 46 species in Hemiptera (22.01%), 24 species in Diptera (11.48%), and 16 species in Hymenoptera (7.66%) respectively. The largest number of species was observed in September, 2010, and the highest was also observed in S4. The diversity index (H') and richness index (RI) of insects at survey area as S4 (H'=3.344, RI=9.257) were higher than in the others and the highest species evenness index(EI) occurred in S1 (0.985). But the lowest was in S3 (H'=2.428, RI=4.307, EI=0.832).

Keywords:*Urban Green Space, Insect Diversity, Community Analysis, Botanic Garden.*

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Ecology

P3

Why we need long-term biodiversity monitoring? A case study of macromoths in Mt. Jirisan, a Korea's Long-Term Ecological site

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Mount Jirisan is the largest (440 ㎢) and highest (1,915 m) national park in mainland of South Korea. The vegetation of mount Jirisan is divided into three main zones: warm temperate (200-300 m in southern aspects), cool temperate (400-1,400 m) and subalpine (> 1,400 m). Macromoths, a major herbivore in forest ecosystem were sampled at six sites to understand ecological effect of vegetation community structure in different elevation. A light trap consisting of a 22-watt ultraviolet light with a 12 V battery was employed once a month from May to October from 2005 to 2010. In total, 20,711 individuals in 922 species of 14 families of macrolepidoptera were collected. Estimated species richness with Chao 1 was 1,105 species. The family Noctuidae (417 species) was the most species-dominant group, followed by Geometridae (307 species) and Notodontidae (59 species). Monthly change in the mean number of species and individuals showed a hump-shaped pattern with the peak at July. Unlike monthly change of richness and abundance, the yearly change was inconsistent: the highest species richness with 504 species was 2008 while the lowest with 359 in 2010; the highest abundance was 5,050 in 2006, while the lowest with 2,376 in 2005. The causal factor for this inconsistent yearly change was unknown. However, the long-term monitoring of moths may reveal the pattern and process of population change of moths according to vegetation and environmental changes.

Keywords:*Moths, herbivore, long-term monitoring*

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PS3M389

Ecology

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Inner-tree distribution of pinewood nematode, *Bursaphelenchus xylophilus* and its vector, *Monochamus saltuarius* in *Pinus koraiensis* in South Korea

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After pine wilt disease (PWD) at Korean white pine, Pinus koraiensis has been first reported in the middle area of South Korea in 2006, it spread rapidly to the nearest area. However, we have little information about biological characteristics and pathological mechanisms of PWD on P. koraiensis compared with PWD on P. densiflora. We selected 14 of dead P. koraiensis in PWD damaged area and analyzed the inner-tree distribution of Bursaphelenchus xylophilus and the entrance holes of Monochamus saltuarius. Bursaphelenchus xylophilus were detected from 12 dead pines. There was no statistical significance between height or direction of wood extraction and the distribution of Bursaphelenchus xylophilus. The entrance holes of M. saltuarius were concentrated on 5 dead pines and the maximum number of entrance holes in individual tree was 543. It is considered that the event of spawning of M. saltuarius adult and entrance of hatched M. saltuarius larvae to wood are dependent on the time of death of host pine trees. The entrance holes were frequently found in a southward direction. That implicated that M. salutuarius larvae prefer relatively weak woody parts to enter easily. The relation between the thickness of bark of P. koraiensis and the frequencies of entrance holes of M. saltuarius was not observed.

Keywords:*pine wilt disease, Pinus koraiensis, Bursaphelenchus xylophilus, Monochamus saltuarius, inner-tree distribution*

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Ecology

P3

Seasonal occurrence of sweetpotato whitefly(*Bemisia tabaci*) and western flower thrips (*Frankliniella occidentalis*) on the pepper greenhouse in Chung-buk Province of Korea

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From 2010 to 2012, 10 institutions have been studying Sporadic and major Insect Pests in Crops to build surveillance system of National Pest Management System(NPMS). This studies were conducted on pepper greenhouse in Goesan-gun, Eumseong-gun and Cheongju-city of Chung-buk Province to investigate the seasonal occurrence of sweetpotato whitefly and western flower thrips. The 5 yellow trap(10×15cm) per site were used every 10 days from May 1 to October 10. In red pepper, the population density of Bemisia tabaci was increased after early-August and the caught number per trap was recorded over 100 adults after early-september. The population density of Frankliniella occidentalis was achieved the peak during early and mid-july and then decreased.

Keywords:*greenhouse pepper, Seasonal occurrence, Bemisia tabaci, Franklinella occidentalis, yellow trap*

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Ecology

P3

Seasonal occurrence of *Metcalfa pruinosa* (Say) in Chungbuk Province and establishment of optimal control timing

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This study was investigated the seasonal occurrence of Metcalfe pruinosa (Say) in Chungbuk province and established the optimal control timing. Population densities of nymph and adults were investigated and egg hatching period and oviposition period was examined from three locations (Cheongwon, Jincheon, Eumseong area). Egg hatching period was investigated. The first day of egg hatching observed on May 25, the day of peak egg hatching was on May 29, and the perfect accumulative egg hatching ratio was on Jun. 13. Oviposition period of M. pruinosa female was observed the cut branch (cut within 10 cm length, 1 cm dia.) of oak tree, an althea, and an acacia under stereoscope. The first oviposition was observed at Jul. 28 and continually increased until late-Sept. with sharply increased at mid-Aug. Population density of M. pruinosa nymph was observed the cut branch (cut within 30 cm length, 1 cm dia.) of oak tree, an althea, an acacia, and pear tree. Population density began decreased showing after highest density with 25.3 (Jul. 1). The adult was first appeared at Jul. 11 and showed highest population density with 13.1 (Aug. 4) and then continuously decreased.

Keywords:*Metcalfe pruinosa, Egg hating period, Oviposition period, Population densities, Optimal control timi*

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Ecology

P3

Stress response to acoustic stimuli in an aphid: A behavioral bioassay model

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The green peach aphids, Myzus persicae (Sulzer), feed consistently and regularly throughout all stages of nymphs and adult. That is, honey dew production (HDP), which indicates a state of feeding, occurs at regular intervals within a given stage, and moving (MV), which interrupts feeding, tends to occur scarcely. We used these behaviors to test whether the sound stimuli acted as a stress in this species. We conducted the playback experiment in which acoustic stimuli with a combination of five frequency (100, 500, 1000, 5000, and 10000 Hz) and three intensity levels (66, 78, 90 dB SPL) were presented to all stages of aphids. Under all acoustic stimuli, HDP occurred less often, and MV occurred more often and longer. The results of two-way multivariate ANOVA showed that the stimulus frequency was a significant factor for variation in aphid behaviors in the playback experiment. However, there was no intensity effect on aphid's behavior. Based on the electrical penetration graph experiment, aphid feeding was frequently stopped and lasted only for short durations under acoustic stimuli. The results of both experiments demonstrated that the acoustic stimuli were effective in inducing feeding suppression in this species. Thus, the acoustic stimuli may be a stressor in the green peach aphid. Furthermore, the behavioral bioassay employed in this study may be used to assess the effect of various stressors on aphids.

Keywords:*Myzus persicae, feeding supression, playback experiment, electrical penetration graph*

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Ecology

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A study on research history and distribution of *Drosophila suzukii*

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Although the damage by the Drosophila suzukii (Spotted wing drosophila) was reported in Incheon in 1935 for the first time in Korea, the damage on fruit farm was not reported afterwards. In the wild conditions excluding fruit farm, reports on the spotted wing drosophila have been continuously made through the local distribution of drosophila. Jung (1958) reported the emergence of the Drosophila suzukii in 10 palces in South Korea, Lee(1987) in Muju-gun in Jeollabuk-do and Gyeryongsan in Chungcheongnam-do, Lee and Kim(1987) in 11 mountains including Mt. Halla, Kim et al.(1992) in Mt. Sori in Gyeonggi-do, Lee et al.(1993) in Mt. Chiak in Gangwon-do, Kim et al. (1997) in Gyeongsan and Jinju Neuk-do area. Additionally, Lee(1966) recorded 48 areas in the nation showing that the Drosophila suzukii is distributed in most of the areas in Korea. This study was performed from September 2011 to July 2012 to identify the distribution of the Drosophila suzukii using Drosophila suzukii traps. From the result, it was found that the Drosophila suzukii emerged in 20 points including Jeju-do and the outbreak time and outbreak concentration of Drosophila suzukii by time were identified.

Keywords:*Drosophila suzukii, SWD, History, Distribution, Korea*

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Ecology

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A study on host plant of *Drosophila suzukii*

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Drosophila suzukii (Spotted wing drosophila) is originated from Japan and is constantly identified in Asia such as China and Korea, North America such as USA and Canada, and Europe such as Italy, France and Spain. In particular, in USA it brought economic damage such as 20% reduction of the harvest of the berries in 10 places in USA after 2008. Drosophila suzukii is identified as the species doing harm on the various fruits including berries. In this study, the host plants of the Drosophila suzukii were identified. The host plants of the Drosophila suzukii known up to now were 40 species in 12 families such as 25 species in Rosaceae, 1 species in Ericaceae, 1 species in Elaeagnaceae, 1 species in Sapindaceae, 2 species in Caprifoliaceae, 3 species in Moraceae, 1 speices in Cornaceae, 1 species in Cornaceae, 3 species in Vitaceae, 1 species in Ebenaceae, 2 species in Actinidiaceae. Based on the information of the aforementioned host plant information, inoculation elements were performed on the grapes, cherries and blueberries cultivated in Korea and the results are reported here.

Keywords:*Drosophila suzukii, SWD, Host plant*

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Ecology

P3

Occurrence patterns, life cycle and its environmental friendly control of *Pochazia* sp. in the Apple Orchard

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Leafhopper wings (Pochazia sp.) are occurred areas at Gong-ju, Ye-san, Gu-re, Soon-chang, Go-yang and throughtout the country from 2009. Apples, persimmons, chestnuts, schisandra chinensises, blueberries, and raspberries etc. are known as host. Leafhopper wings which are pests with mouth types for sucking mainly suck sap from leaves and branches of woody plants. After hatching in late May, nymphs were at the end of July and in early September adults were the peak occurrence. Damage by oviposit is more serious than feeding damage by nymphs and adults in apple orchards. Because the adult females through ovipositor lay eggs in old branches after stripped its barks. In addition, according to survey for life history during indoor rearing at 25℃, developmental periods of each instar took average 9.6 days and adults took average 51 days from egg stage. The environmental friendly control was used plant extracts that were toosandanine, oxymathrin, eugenol, veratrine, rotenone, matrine, neem oil, tobacco leaf and emodin. Active ingredient was 0.3%, and diluted 500, 1000 and 2000 times for use. Extracts of matrine and rotenone were showed a relatively high insecticidal activity against young nymphs.

Keywords:*Pochazia sp., life cycle, plant extract, environmentally-friendly control*

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Ecology

P3

A study on the conservation of butterflies in South Korea included in IUCN Redlist

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The IUCN composed the SSC in 1949 and thereby grasped the extinction and reduction of species existing on earth and the causes and set a guideline for the scientific and objective selection of endangered species. It framed the Redlist by classifying the extinct or endangered species with that as the criteria and is recommending protecting them worldwide. However, Korea only has designated the national endangered species and is protecting and managing them, and the protection of butterflies in Korea included in IUCN Redlist has not been conducted yet. Therefore, this study aims to grasp the present condition of distribution of the butterflies in Korea included in the IUCN Redlist and to present a plan for conservation of them in Korea as they are endangered worldwide. The samples possessed by 22 colleges and institutions in Korea were examined since March, 2008 until August, 2009 in order to investigate the records of distribution from the past. The recorded species in the Korean Peninsula belonging to the Redlist of IUCN are 5 species in total of Coenonympha oedippus, Maculinea teleius, Maculinea cyanecula, Maculinea arionides and Lycaena dispar and are classified as the stage of Lower Risk/near threatened. Maculinea cyanecula was excluded from this research because this species habitats are restricted to North Korea only. As a result of the research on the 4 species, 12 individuals of Maculinea teleius, 25 individuals of Coenonympha oedippus, 52 individuals of Maculinea arionides, and 333 individuals of Lycaena dispar were identified.

Keywords:*IUCN, Redlist, Butterfly, Distribution, Korea*

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Ecology

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An integrative approach to species delimitation in three closely related cricket species (Genus *Loxoblemmus*)

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Selection against costly reproductive interaction is most likely to occur between closely related species in sympatry. Then, the divergence in sexual traits for mate recognition is expected to be more conspicuous than are divergences in neutral traits such as other morphological characteristics or molecular sequences. Cricket songs are species-specific and are used for species recognition and mate choice. Three Loxoblemmus cricket species occur sympatrically throughout Korea: L. doenitzi, L. campestris and L. equestris. The horn-headed cricket, L. doenitzi, is morphologically distinguished from the other two species, whereas L. campestris and L. equestris are morphologically very similar. We predicted that calling songs of L. campestris and L. equestris were more differentiated from each other than each from that of L. doenitzi. To test this prediction, we used an integrative approach by comparing the divergences of three types of data: morphology, calling song, and genetic sequence. Because there was no reliable morphological character for species identification, we started the analyses with L. doenitzi and the L. campestris/equestris complex. As expected, the distributions of measured morphological characters did not discriminate between L. campestris and L. equestris. However, the analysis of calling songs clearly separated the L. campestris/equestris complex into two groups. Furthermore, the divergence of mitochondrial COI was consistent with that of calling songs in the L. campestris/equestris complex. The genetic sequence analyses revealed that L. doenitzi diverged off earlier from the other two species. These results suggest that processes of sexual selection may have driven the speciation in the Loxoblemmus complex.

Keywords:*calling song, reproductive interaction, sexual selection, species recognition*

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Ecology

P3

Population genetics study of the lantern fly, *Lycorma delicatula* (Hemiptera: Fulgoridae): Invasion route from China to Korea

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Lycorma delicatula (White 1845), which has been recently introduced into Korea, is a notorious pest on grapes. This invasive insect has rapidly spread throughout central and southern Korea. To compare genetic characteristic and differentiation between Lycorma delicatula populations, we collected samples in East Asia. Between 2010 and 2011, we collected totally ca. 800 individuals in 36 populations from Beijing, Tianjin, Shandong, Jiangsu, Shanghai, Zhejiang in China, and from Ishikawa in Japan. First we analyzed representative populations in Korea, China, and Japan using ND2 and ND6 sequences by neighbor-joining method. As results, the populations of Shanghai, Beijing, Tianjin, and all Korean regions shared a same haplotype. Second we analyzed population genetics using 12 microsatellites. PCA and STRUCTURE tests showed that Shanghai likely is the origin of the Korean L. delicatula populations. Based on GeneClass2 assignment results, Seoul and Incheon in capital area, and Nonsan and Buan in central area were inferred to the first invading regions within Korea, from where L. delicatula then dispersed to other regions. We tested four scenarios to confirm the invasion route using Approximate Bayesian Computations. As results, L. delicatula originated from Shanghai invaded into both capital and central areas at the same time. In addition, we tested 762 individuals combining the former dataset with additional samples collected in 2006-2009. The structure analysis resulted in that L. delicatula populations occurring in Korea independently were introduced at least three times.

Keywords:*exotic pest, Fulgoridae, invasion, microsatellite, population genetics*

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Ecology

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Population genetic structure of the soybean aphid from Asia and North America based on microsatellites

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The soybean aphid, Aphis glycines Matsumura, was recently introduced from Asia into North America (NA) where it has become a serious pest of soybeans. This invasive pest has rapidly spread throughout the midwestern United States and southern Canada since 2000. We examined 593 individuals obtained from 24 different collections in USA, Korea, China, and Japan using eight microsatellite loci. Based on analysis of multilocus genotype, gene diversity and number of alleles in NA were averaging 0.40 and 2.70, whereas in Asia averaging 0.55 and 4.32, respectively. The factorial correspondence analysis displayed that some Korean populations were closely related to the NA populations. Structure analysis resulted in two conspicuous clusters, NA and Asia, as the most likelihood number of clusters (K=2). Bayesian assignment tests revealed that Osan and Milyang populations in Korea were most likely assigned to the NA populations. Bottleneck test did not show significance of genetic bottleneck in all populations. We also discuss the invasive history of the soybean aphid in light of population genetics.

Keywords:*aphid, Aphis glycines, invasive species, microsatellite, population genetics*

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Ecology

P3

Foliar aphid feeding recruits rhizosphere bacteria and primes plant immunity against pathogenic and non- pathogenic bacteria in pepper

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Plants modulate defence signalling networks in response to different biotic stresses. The present study evaluated the effect of a phloem-sucking aphid on plant defence mechanisms during subsequent pathogen attacks on leaves and rhizosphere bacteria on roots. Plants were pretreated with aphids and/or the chemical trigger benzothiadiazol (BTH) seven days before being challenged with two pathogenic bacteria, Xanthomonas axonopodis pv. vesicatoria (Xav) as a compatible pathogen and X. axonopodis pv. glycines (Xag) as an incompatible (nonhost) pathogen. The aphid population was reduced by BTH pretreatment and by combination treatment with BTH and bacterial pathogens in a synergistic manner. Analysis of the expression of the defence-related genes Capsicum annum pathogenesis-related gene 9, chitinase 2, SAR8.2 and lipoxygenase1 revealed that aphid infestation resulted in the priming of the systemic defence responses against compatible and incompatible pathogens. Conversely, pre-challenge with the compatible pathogen Xav on pepper leaves significantly reduced aphid numbers. Aphid infestation increased the population of the beneficial Bacillus subtilis GB03 but reduced that of the pathogenic Ralstonia solanacearum SL1931. The expression of defence-related genes in the root and leaf after aphid feeding indicated that the aboveground aphid infestation elicited salicylic acid and jasmonic acid signalling throughout the whole plant. The findings of this study show that aphid feeding elicits plant resistance responses and attracts beneficial bacterial populations to help the plant cope with subsequent pathogen attacks.

Keywords:*aboveground, aphid, belowground, PGPR, Xanthomonas axonopodis*

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Ecology

P3

Life table-based computer simulation of population dynamic of *Spodoptera litura*. (F.) fed on groundnut (Arachis hypogaea) leaves under different condition

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Spodoptera litura (F.) is the most destructive leaf-defoliating pest of peanuts (Arachis hypogaea) in Taiwan. To accurately simulate the population dynamics of S. litura, life table data, using the age-stage, two-sex life table, were collected at constant temperature 25ºC in the laboratory, and outdoors under field conditions in the spring and fall of 2010. Results showed that there were significant differences in developmental times, survival rate, fecundity, and hatch rate under different conditions. The shortest immature stage occurred in fall (19.2 d), while the longest was in spring (28.4 d). At 25℃, both male and female adults lived longer than adults under field conditions. The mean generation time was the shortest in fall (34.5 d). The preadult survival rates in spring (39.9%) and fall (53.3%) were lower than the rate observed at 25℃ (84.6%). The mean fecundity of S. litura was 3548, 3452, and 3072, at 25℃, in spring, and fall, respectively. The hatch rates were 64.2, 72.2, and 26.0%, respectively. At 25°C, the intrinsic rate of increase (0.1838 d-1), finite rate of increase (1.2017 d-1), and net reproductive rate (963.2 offspring) were the highest among all treatments. Population parameters showed high variability in spring and fall. Because both hatch and survival rates in the field were affected by various unregulated environmental factors, they were lower than those obtained at constant temperature. Our results demonstrate that computer simulation based on life tables collected under both laboratory and field conditions is an important tool for prediction pest emergence.

Keywords:*Spodoptera litura, Arachis hypogaea, tow-sex age-stage life table, population simulation*

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Ecology

P3

Life table of *Kolla paulula* (Walker) reared on *Bidens pilosa* L. var. radiata Sch. and *Wedelia triloba* (L.)

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Kolla paulula (Walker), the xylophagous leafhopper, was recently identified at the Taiwan Agricultural Research Institute as a potential vector of the xylem-limited bacterium, Xylella fastidiosa (Wells), the causative agent of Pierce's disease and pear leaf scorch disease in Taiwan. The developmental rate, survivorship, reproduction, and life-table parameters of K. paulula reared on mixture of Pilose beggarticks (Bidens pilosa var. radiata) and Trilobate Wedelia (Wedelia triloba L.) were studied using the age-stage, two-sex life table. Experiments were carried out outdoors under naturally fluctuating temperatures from Feb. to Aug. 2011. The total immature development time was 53.0± 4.0 d. The survival rate was observed in the egg stage (81.6%), while the survival rate of other stages was as high as 98%. The adult preoviposition period was 6.9 d, and the mean fecundity was 91.5 ± 39.3 eggs. The adults could survive, on average, 1.5 mo (46.8 ± 16.3 d for females and 50.3 ± 16.7 d for males). The intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate(Ro), and mean generation time (T) were 0.0473 d-1, 1.0485 d-1, 33.7 offspring, and 74.3 d, respectively.

Keywords:*Kolla paulula, xylem feeders, life table, population parameter*

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Ecology

P3

Locality records of potato tuber moth, *Phthorimaea operculella* (Zeller), distributed in the Korean Peninsula

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Potato tuber moth (PTM), Phthorimaea operculella (Zeller), is a cosmopolitan pest, especially in warm temperature and tropical regions where host plants, mostly belong to family Solanacea, are grown. In recent years, the species has been inadvertently occurred in the potato fields of middle areas of Korea. To determine the distribution of PTM, we carried out an extensive survey using sex pheromone traps at 38 sites across the nation during 2009~2011. Delta-typed traps were baited with rubber septa impregnated with commercially available PTM sex pheromone. In each area surveyed, a potato field approximately 0.1~3 ha in size was selected and 2 traps were randomly placed in that field 1 m above ground level. As a result, we confirmed that the range of PTM extended from southern coastal regions to the central regions of the Korean Peninsula. The northern limit of PTM distribution expanded to northern Gyeonggi (Yeoncheon, Gangwha, Gimpo, Paju) and middle Gangwon regions (Goseong, Inje, Pyeongchang) from Gyeongbuk and Chungbuk regions reported first by Choi & Park (1980). Based on these data, we investigated why drastic going northward of PTM happened in Korea. Here is one presumption that a sudden rise in average temperature has occurred during 30 years, from 6.34℃ of 1973 to 7.62℃ of 2007, in the Korean Peninsula.

Keywords:*Phthorimaea operculella, Solanum tuberosum, distribution, Korea*

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Ecology

P3

Investigation the effect of storage of wheat and corn on Mediterranean flour moth based on an Age-Stage, Two-Sex Life Table

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However Ephestia kuehniella is a cosmopolitan pest of stored products, its eggs and larvae are widely used to rear parasitoids and predators. Therefore investigation the effect of storage of wheat and corn on this insect is important particularly economically for mass rearing in insectariums. Most life-table analyses have been concerned only with the female population, however, this moth is not parthenogenetic and both males and females are economically important. Treatments used in this study include equal proportions of corn and wheat flour (48.5%) and 3% yeast: 1- new corn, new wheat; 2- new corn, old wheat;3- old corn, new wheat; 4- old corn, old wheat. Experiment was carried out with 50 replications at controlled conditions. Data were analyzed based on an age-stage, two sex life table model developed by Chi. The results indicate that there is significant difference in mean generation time (T) and the most and the least amount of T respectively were in treatment 2 and 3. But there was no significant difference in other parameters such as the gross reproduction rate (GRR) and the net reproduction rate (R0). Although treatments had different the intrinsic rate of increase (rm) no significant difference was observed. Also the most weight of larvae, pupa and adult and the number of eggs and The lowest percentage of deaths and adult longevity occurred in treatment 1. When the cost is taken into consideration, new corn and wheat is recommended to produce satisfactory quantity and quality of E. kuehniella adults and eggs.

Keywords:*Ephestia kuehniella, Age-stage two-sex life table, diet*

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Ecology

P3

Review of the food plants of Lepidopteran larvae outbreaking in a national park and outbreaking species management

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In this study, lepidopteran larvae outbreaking in a national park and the food plants of these larvae were identified to determine proper management methods. Numerous trees on climbing paths near Donghak Temple and Gap Temple at Mt. Gyeryong National Park were investigated from April to June in 2009, 2010, and 2011. In the survey, it was found that the three species of Alcis angulifera, Cosmia affinis, and Libythea celtis outbreak and utilize multiple food plants in addition to their well-known food plants as noted in the literature. In the case of A. angulifera, it eats a total of 12 species not only of previously known food plants such as Quercus serrata, Weigela subsessilis, Castanea crenata, Celtis sinensis, and Camellia japonica but also Zelkova serrata, Styrax japonicus, and Prunus sargentii. It was also found that C. affinis and L. celtis each had seven species of food plants. The survey results show that they are polyphagous insects that eat a wider range of food plants than previously known. The reasons why insect outbreaks occur remain unclear. They arise, however, when multiple elements are combined and when the diversity of the food plants has a very close relationship with the outbreaking insect. As insect outbreaks generally have detrimental effects on plants and on other species, often disturbing the natural ecosystem, most countries consider outbreaks to be a very sensitive issue. At the same time, in advanced countries such as the USA and in Korea, outbreaks arising in national parks are a major concern.

Keywords:*Outbreak, Lepidopteran larva, Food plant, National park, Management*

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Ecology

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Brood size and sex ratio regulation in the pupal ectoparasitoid, *Pteromalus apum* parasitizing the Glanville fritillary butterfly, *Melitaea cinxia*

Norio Hirai[1], Saskya Van Nouhuys[2]

[1]*Osaka Prefecture University, Japan,* [2]*University of Helsinki, Finland*

Pteromalus apum is a gregarious pupal parasitoid of butterflies, including the Glanville fritillary butterfly, Melitaea cinxia. We investigated the brood size and sex ratio under 4 different superparasitism situations using M. cinxia pupae as a host. We exposed 1, 2 or 3 female wasps to a host. When a host was exposed to female wasps sequentially, each for 1 hour, the brood size increased with number of females used. The proportion of offspring that was male was 5-7%, with no significant difference between the 3 treatments. Similar trends were observed when a host was exposed to each female wasp for 1 hour, with a 24 h between each. When a host was exposed to 2-3 female wasps simultaneously for 24 hour, both brood size and proportion of the brood that was male increased with the number of females used. Similar results were also obtained when we use Inachis io and Aglais urticae as hosts. Thus, the sex ratios from singly parasitized hosts were strongly biased towards females and did not differ among all the sequential oviposition experiments. But when multiple wasps were with a host at the same time the sex ratio of the progeny increased. This suggests that physical contact among female wasps is primarily responsible for the sex ratio response in this species.

Keywords:*parasitoid wasp, sex ratio, oviposition, butterfly, host-parasitoid relationship*

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Ecology

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Mosquito fauna in Singapore

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The Republic of Singapore is a tropical island-city situated 137 kilometres north of the Equator. It comprises of mainland Singapore and its 62 islets. Despite extensive urbanisation, approximately 23% of its land area still remains forested. As a result, Singapore has an abundance of diverse fauna of mosquito where 125 species were reported in 1986. From 2003, Environmental Health Institute (EHI), the mosquito reference centre of Singapore, worked with various stakeholders to collect adults and larvae routinely from vegetated areas on the main and other smaller islands. The information is compiled in a database that is invaluable to our understanding of the diversity, distribution and seasonal trends of mosquitoes. At the same time, specimens (larvae and adults) were carefully curated in EHI. In 2010, 12 species including nine new records were added to this list. Till date, EHI has a collection of over 7000 adults and 900 larval specimens, representing at least 80 mosquito species. This represents the most comprehensive mosquito collection in Singapore.

Keywords:*Culicidae, diversity, distribution, reference centre*

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Ecology

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Responses of Tabanidae (Insecta: Diptera) species to Malaise traps baited with octenol

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Adult female Tabanidae species which are blood sucking parasites cause serious problems in terms of medical and veterinary. No fully-effective methods are mentioned on literature to keep Tabanidae species under control. Synthetic and natural attractant including traps are in use on many parts of the world to collect female tabanids. Many attractants may be effective or ineffective under various environmental conditions in different geographic regions for horseflies. Octanol has a effect on many horsefly species behaviours and when used with other phenolic compounds it becomes very effective for this group of insects.. The study was performed on Catacık forests and Hekimda? in Eski?ehir and it emphasize the relationship between traps baited with the octanol and control traps. The results present that Tabanus is the most represented genus with 93,6 % of the total collected tabanids. The chi-squared analyses show that traps baited with the octanol collected 5 times more tabanids than control traps and Tabanus briani (Leclercq, 1962), Tabanus rupium (Brauer 1880) and Tabanus portschinskii (Olsufjev, 1937) species are only determined on the octanol baited traps. Research results were evaluated by comparing with other studies made with octanol. As a result, female tabanidleri to control or minimize their losses in order that there may be an effective method of using with octanol traps.

Keywords:*Diptera, Tabanidae, Octanol, Eski?ehir, Turkey*

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Ecology

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First record in Egypt of two thrips species infesting cucumber crops

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[1]Fac. of Agric.-Cairo University, Egypt, [2]Fac. of Agric.-Cairo University, Egypt, [3]Fac. of Agric.-Cairo University, Egypt, [4]Fac. of Agric.-Cairo University, Egypt

Field survey was made in four successive cucumber plantations (sown on mid April and mid August, 2010/2011) in Giza, Egypt. Direct inspection of weekly leaf samples revealed that two thrips species Chirothrips texanus Andre and Thrips palmi Karny were detected for the first time in Egypt, in addition to Thrips tabaci Lindeman and Frankliniella occidentalis (Pergande). The highest number of thrips species was recorded in August plantations for Thrips tabaci (923 individuals/25 leaves) followed by Ch. texanus (679 individuals/25 leaves). F.occidentalis recorded the lowest number (368 individuals/25 leaves). The present finding updates the list of thrips species in Egypt.

Keywords:*Chirothrips texanus, cucumber, Thrips, Thrips palmi*

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Ecology

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Sequential sampling and population dynamics of *Rhopalosiphum maidis* and *Schizaphis graminum* aphids in Badjgah region (Fars province)

Vajiheh Soltani Ghasemloo
Iran

SEQUENTIAL SAMPLING AND POPULATION DYNAMICS OF Rhopalosiphum maidis and Schizaphis graminum APHIDS IN BADJGAH REGION (FARS PROVINCE) Vajiheh Soltani Ghasemloo1, Maryam Aleosfoor2, Abdolamir Mohiseni3, Mahmood Alichi2, Lida Fekrat4 1College of Agriculture, Shiraz University, Shiraz, Iran. E-mail: v_soltani84@yahoo.com , 2Associated Professors of Plant Protection Department, College of Agriculture, Shiraz University, Shiraz, Iran. 3Associated Professors, Agricultural and Natural Resource Research Center of Lorestan, 4Associated Professors of Plant Protection Department, College of Agriculture, Ferdowsi University, Mashhad, Iran During 2010-2011, fixed precision sequential sampling plans with X shape pattern of movement in the field were investigated for two aphids Rhopalosiphum maidis and Schizaphis graminum, in fields of wheat in Badjgah (Fars province). Based on Dancan test, and RV, the best sample unit was 4 stems. taylor's power law gave a suitable fitness. Since regression line slopes weren't significantly different for S. graminum and R. maidis, comparing both of them by the same Green model was proposed. In precisions of 0.15, 0.25 and 0.3 in Green's fixed- precision method, minimum numbers of samples for R. maidis and S. graminum were 20, 12 and 9, respectively. The numbers of needed samples in Green method with 0.15, 0.25 and 0.3 precision were reduced to 77.8±1.05, 78.3±0.91 and 81.4±0.81, respectively. When the population mean of these aphid was 1.6, 3.86 and 5.62 poroportion of infection in the field was 0.5, 0.75 and 0.85, respectively. Relationship between sample number and plant with aphids showed that sample number reduce with increasing in infection percentage.

Keywords:*aphid, Rhopalosiphum maidis, Schizaphis graminum*

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Ecology

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Determining the best sample unit in sequential sampling for *Rhopalosiphum padi* (L.) in Badjgah wheat field

Vajiheh Soltani Ghasemloo
Iran

Determining the best sample unit in sequential sampling for Rhopalosiphum padi (L.) in Badjgah wheat field Vajiheh Soltani Ghasemloo1, Maryam Aleosfoor2, Abdolamir Mohiseni3, Mahmood Alichi2, Lida Fekrat4 1College of Agriculture, Shiraz University, Shiraz, Iran. E-mail: v_soltani84@yahoo.com , 2Associated Professors of Plant Protection Department, College of Agriculture, Shiraz University, Shiraz, Iran. 3Associated Professors, Agricultural and Natural Resource Research Center of Lorestan, 4Associated Professors of Plant Protection Department, College of Agriculture, Ferdowsi University, Mashhad, Iran Rhopalosiphum padi is one of the most important aphids in wheat fields of Iran. During 2010-2011, population fluctuation, spatial distribution and fixed precision sequential sampling plans of this aphid was investigated in fields of wheat in Badjgah (Fars province). This aphid attacked wheat fields in the late April and its population peak was seen on April 9th 2011, when the mean temperature and relative humidity were 11.25 ?C and 33.5±5%, respectively. RV results in the fields (bahar cultivar), for 1,2,3,4 and 5 stems calculated 38.1,32.9,29.3,28.7 and 28.62, respectively. 4 and 5 stems did not show any significant difference. The results of RNP analysis for 1,2,3,4 and 5 stems were 6.7,6.1,5.6,5 and 4.5. RV showed that 4 stems was the best choicein proportion 5 stems because it consumed lower time for counting aphids. High RNP showed high efficiency in number of sample or sampling method, but in this project 1 stem had highest RNP, so we can not accept it. At the end the best choice for sampling unit was 4 stems.

Keywords:*Rhopalosiphum padi,aphid*

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Sequential sampling and Green?model with Wilson & room's model for *Sitobion avenae* in Badjgah fields (Fars Province)

Vajiheh Soltani Ghasemloo
Iran

Sequential sampling and Green' model with Wilson & room 's model for Sitobion avenae in Badjgah fields (Fars Province) Vajiheh Soltani Ghasemloo1, Maryam Aleosfoor2, Abdolamir Mohiseni3, Mahmood Alichi2, Lida Fekrat4 1College of Agriculture, Shiraz University, Shiraz, Iran. E-mail: v_soltani84@yahoo.com , 2Associated Professors of Plant Protection Department, College of Agriculture, Shiraz University, Shiraz, Iran. 3Associated Professors, Agricultural and Natural Resource Research Center of Lorestan, 4Associated Professors of Plant Protection Department, College of Agriculture, Ferdowsi University, Mashhad, Iran During 2010-2011, Sitobion avenae was one of the most important aphid in wheat fields of Iran . Fixed precision sequential sampling plans of this aphid was investigated in fields of wheat in Shiraz. Taylor's power law and Iwao's index were used to evaluate spatial distribution of the aphid. Results showed that taylor's power law gave a better fitness for the aphid in comparison with Iwao's index. Green's? sequential sampling plans were designed for estimating population density at three fixed precision levels (0.15, 0.25 and 03). When aphid mean in sample unit was 0.52,1.25 and 1.48, the proportion of infection was 0.28, 0.59 and 0.64, respectively. When the proportion of infection was 0.61, in decision levels 0.15, 0.25 and 0.3 the sample's number was 122, 24 and 17, respectively.

Keywords:*Scitobion avenae,aphid*

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Ecology

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Preference of the natal fruit fly, *Ceratitis rosa* to different fruit scents in a choice trial

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Ceratitis rosa (Natal Fruit Fly) is one of the most economically important pests of citrus fruits throughout the world. The status of C. rosa like other fruit flies as a major pest, is maintained on cultivated fruits because it is more abundant in both cultivated and wild fruits throughout the year. C. rosa has a wide range of hosts including citruses as one of the most preferred cultivated hosts. Fruit fly management aims at deploying Bait Application Technique (BAT) which has been considered to control fruit flies over a large area. This can be achieved by using an "Attract and Kill" approach incorporating attractants in the form of food and pheromones. In this study fruit scents have be used as attractants in a bait station to attract flies to the killing agent. Flies have been introduced to the different fruit scents in a choice experiment to determine the most preferred scent/s and preliminary results have shown that orange scent (Limonene) and banana scent (Isomyl acetate) are the most attractive to the Natal fruit fly. Further trials will be carried out to further test the attractiveness of these scents in fruit fly baits.

Keywords:*C. rosa, Biocontrol, B. bassiana, fruit flies*

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Ecology
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Quantifying dispersal of *Trichogramma brassicae* in the tomato fields using diffusion model

Samaneh Soltantoieh[1], Gholamhossein Gharekhanig[2], Ali Mehrvar[3]
[1]University of Maragheh, Iran, [2]University of Maragheh, Iran, [3]University of Shahid Madani, Iran

The study of insect dispersal plays an essential role in estimating the aerial spread of damage caused by a newly invaded pest or the spatial distribution of an useful insect during the active period. Although plenty of studies on trichogramma has been focused on potential of this parasitoids, quantitative knowledge of the movement of trichogramma species is lacking in the field. To fill this gap we intended to study Trichogramma brassicae dispersal using diffusion model. The parasitoids were reared on grain moth eggs, marked using fluorescent powder and released from a central point at 12 different dates from fruit set to harvest and repeated five times. Trap cards were used at distances including 2, 5, 9, 12, 18 and 25m and height 20 Cm from a central releasing point. Parasitized eggs were collected and examined three and six days after release respectively. Non-linear regression was used for estimating the parameters and fitting the models. Accuracy of the estimated parameters was tested according to the default asymptotic 95 % confidence intervals. Indirect method was used for estimating diffusion coefficient (D), R2, migration and mortality (K) and population size (Y) three days after releases. Estimate ranged for (D) from 5.0322-19.2901, R2= 89.2, (K) from 0.0036-0.0976 and (Y) from 1869.14 - 4357.12. Resulted models were compared for different dates. Results revealed the fitness of models for trichogramma dispersal at different dates and for overall experimental time as well as suitability of diffusion model for study of trichogramma dispersal behavior.

Keywords:*Disprsal, Diffusion,Quantifying, Trichogramma, Tomato*

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Ecology

P3

A study of the community structures of the dead wood dwelling arthropods

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The dead wood?dwelling arthropod communities were surveyed from 2010 April to 2011 October in three areas (Mt. Woonak, Mt. Wolchul, Mt. Jingang). Arthropods were sampled with an vacuum aspirator. Arthropod species were classified regarding their functional association as herbivores, fungivores, xylophagous, saproxylophagous, detritivores, and predators. Arthropod communities with 5 variables were analyzed. We collected 8,792 arthropods during our study (5 classes, 20 orders, 58 families, and 93 species). But larva was not counted. The species richness and abundance of arthropods were increased along with the progress of the decay stages for the conifer and deciduous. The species richness index was increased and the evenness index was shown to be at a lower value at the late decay stage than at the other decay stages. The diversity index (H') in conifer was lower than the deciduous at the early decay stage. The deciduous was lower than the conifer at the decay stage. Arthropod communities of functional groups, except the xylophagous were not different in the variables. Xylophagous increased as the decay stages progressed. The abundance of arthropods and xylophagous were significantly different statistically. Overall, the logs play an important role for arthropods as habitats. Thus, we realize that the dead woods are important as a component factor in the forest. When we come up with forest management measures with these important facts, so that dead wood has a good role in the forest, the arthropod community, the species biodiversity of arthropods and higher predator biodiversity will be more complex and maintainable.

Keywords:*Dead woods, Arthropods, Decay stages, Functional groups, Active methods*

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Ecology

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A predictive model for the spring emergence of the pine needle gall midge *Thecodiplosis japonensis* (Diptera: Cecidomyiidae): Applicability of empirical predictive model based on field observations

Youngwoo Nam[1], Mun-Jang Jeon[2], Won Il Choi[3], Sang-Hyun Koh[4]

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Empirical models based on field observation are often better in practice for predicting field events although phenology models are usually constructed using laboratory temperature-dependent development data. Based field observation in Yeongcheon, Korea from 1986 to 2010, the empirical predictive model for spring emergence of Thecodiplosis japonensis (Diptera: Cecidomyiidae) was constructed. Spring emergence of T. japonensis adults occurred from mid-May to mid-July at Yeongcheon and showed wide spread patterns with annual variation during 1986-2010. The correlation between pooled cumulative proportion of spring emergence versus cumulative degree days, using a selected baseline temperature, was fitted with a Weibull function. By varying the baseline temperature (from 3 to 9 °C at 0.5 °C increments), it was determined that the use of 6.1 °C as the lower temperature threshold of its post-diapause development gave the highest R2, indicating that 6.1 °C was likely to be the lower temperature threshold of T. japonensis. The lower temperature threshold based on field observations was only 0.2 °C higher than that based on laboratory temperature-dependent development data (Son et al. 2007). Also, both degree-day models using the lower temperature threshold based on laboratory and field observation accurately predicted spring emergence of T. japonensis in 2011, suggesting that that estimation of the degree-days needed for post-diapause development could be based on field observations and would be a useful approach to develop a phenology model.

Keywords:*degree-day model, spring emergence, Weibull function, Thecodiplosis japonensis, phenology*

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Ecology

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Effect of temperature on development of *Elcysma westwoodi* (Lepidoptera: Zygaenidae) in Korea

Sung-Jae Jeon[1], Sang-Hyun Koh[2], Youngwoo Nam[3], Sang-Gil Lee[4]

[1]Korea Forest Research Institute, Republic of Korea, [2]Korea Forest Research Institute, Republic of Korea, [3]Korea Forest Research Institute, Republic of Korea, [4]Korea Forest Research Institute, Republic of Korea

A tailed zygaenid moth, Elcysma westwoodi (Vollenhoven) has a univoltine with a winter diapause as young larval instars and a summer diapause as prepupa in a cocoon. Temperature has been known to be one of the major regulatory factors of development and initiation of the summer diapause. This study presents a comprehensive summary of development, mortality, and initiation of summer diapause in relation to temperature. The larvae collected in the field divided into three groups based on the distribution of headcapsule widths, which assumed second instar in group A, third instar in group B and forth instar in group C. Regardless of group classification, developmental rates generally increased with increasing temperature from 15 to 25 °C and rapidly decreased to zero at 30 °C. Also, mortality rate for all groups was lowest at 20-25 °C and were 100 % at 30 °C. These results suggest that 25 and 30 °C are the optimum temperature and higher threshold temperature on post-diapause development, respectively. The developmental periods from overwintered larva to initiation of the summer diapause in each groups ranged from 85.5 days at 15 °C to 46.5 days at 27 °C for group A, from 80.3 days at 15 °C to 45.6 days at 27 °C for group B and 74.2 days at 15 °C to 41.9 days at 27 °C for group C. These results suggest that temperature plays an important role in life history traits of E. westwoodi through not only temperature-dependent development, but also through high-temperature inhibition.

Keywords:*Elcysma westwoodi, temperature-dependent, summer diapause, phenology, Zygaenidae*

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Ecology

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Binomial sampling plans for *Aphis gossypii* (Hemiptera: Aphididae) on greenhouse cucumbers

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[1]Institute of Horticultural and Herbal Science, RDA, Republic of Korea, [2]Seoul National University, Republic of Korea, [3]Korea University, Republic of Korea, [4]Korea University, Republic of Korea

Infestations of Aphis gossypii per leaf on commercial greenhouse cucumbers were determined for developing binomial sampling plans. An empirical PT-m model, , was used to evaluate relationship between the proportion of infested leaves ≤ T aphids per leaf (PT) and mean densities (m). Tally thresholds (T) were set to 1, 3, 5, 7 and 9 aphids per leaf to find appropriate T in cucumber greenhouse system, respectively. Increasing sample size did little effects on the precision of the binomial sampling plan. However, the precision increased with higher tally threshold. The binomial model with T = 5 provided appropriate predictions of mean densities of A. gossypii on greenhouse cucumbers. By results of the binomial model with T = 5 (sample size = 200), wide ranges of densities (1.2 - 222.8 aphids per leaf) could be estimated with precision levels of 0.346 - 0.380 for PT values between 0.15 and 0.96. Binomial models were validated at T = 5 and 7 using twelve independent data sets on different greenhouse cucumbers. Both binomial models were robust and adequately described most of independent sampling data fell within 95% confidence intervals around the prediction model.

Keywords:*Cotton aphid, Aphis gossypii, binomial sampling, tally threshold, cucumber*

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Ecology

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Molecular operational taxonomic units (MOTUs) of chironomids (Diptera: Chironomidae) for water quality assessment in Korea

Tae Joong Yoon[1], Min Jeong Baek[2], Jeong Mi Hwang[3], Won Young Choi[4], Yeon Jae Bae[5]

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The non-biting midge family Chironomidae is one of the most widely distributed and abundant group of organisms in diverse freshwater habitats. The composition of chironomid larvae has been frequently used as biological indicators of water quality because a wide spectrum of responses to environmental stress is observed. However, there is a fundamental problem with the use of chironomids because species level identification by using chironomid larval morphology is virtually impossible. Therefore, it is suggested that the molecular operational taxonomic units (MOTUs) equivalent to the traditional species level identification could be used for this purpose. This study was conducted to investigate the relationship between MOTUs and the water quality of streams in Korea. The chironomid larvae were collected from diverse reaches in streams and rivers that showed different levels of water quality. The partial mitochondrial DNA cytochrome c oxidase I (COI) genes (ca. 670 bp) were sequenced and analyzed in relation to the water quality. The results showed a close correlation between the chironomid MOTUs and chemical water quality, thus providing species-specific information about the stream environment.

Keywords:*Chironomidae, cytochrome c oxidase I (COI) gene, molecular operational taxonomic unit (MOTU), biological water quality assessment*

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Ecology

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Longevity and Life table of the striped fruit fly (*Bactrocera scutellata*) adults on pumpkin flowers

Sung-Wook Jeon[1], Myoung-Rae Cho[2], Taek-Jun Kang[3], Hyeong-Hwan Kim[4], Seung-Jun An[5], Hae Woong Park[6]

[1]National Institute of Horticultural & Herbal Science, Republic of Korea, [2]National Institute of Horticultural & Herbal Science, Republic of Korea, [3]National Institute of Horticultural & Herbal Science, Republic of Korea, [4]National Institute of Horticultural & Herbal Science, Republic of Korea, [5]National Institute of Horticultural & Herbal Science, Republic of Korea, [6]National Institute of Horticultural & Herbal Science, Republic of Korea

Adult longevity and fecundity of the Striped Fruit Fly, Bactrocera scutellata, were studied at 15~33oC with 65% RH and 16L:8D in laboratory. The highest values of longevity was observed at 21oC with the values of 138.0 for male and 131.2 for female, followed by 24oC and 27oC. Adult females of the Striped fruit flies did not oviposit at all below 18oC or over 33oC. The highest value of fecundity was observed at 24℃, being 111.4 eggs. Jacknife life was evaluated from eggs to adults of the Striped fruit flies at 4 different temperatures. There was a negative correlation between Net reproductive rate(Ro) and temperature. Net reproductive rate(Ro) decreased with increasing temperatures, being highest at 21oC. In case of the intrinsic rate of increase per day(rm) and the finite rate of increase per day(λ), there was little difference among temperatures. However, doubling time(Dt) and the mean generation time(To) decreased with increasing temperature.

Keywords:*Fecundity, Net reproduction rate, Intrinsic rate of increase per day, Doubling time, Mean generation*

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Ecology

P3

Mating behavior and attractants of the pumpkin fruit fly, Bactrocera depressa (Tephritidae: Diptera)

Seung-Joon Ahn[1], Kyung San Choi[2], Taek Jun Kang[3], Sung-Wook Jeon[4], Dong-Soon Kim[5]

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The pumpkin fruit fly, Bactrocera depressa (Tephritidae: Diptera) is one of the most important pests in Cucurbitaceae plants. The mating behavior of B. depressa consisted of: (1) approach, (2) turning, (3) jumping or striking, and (4) copulation. Volatiles that were collected from males during mating showed two active peaks in GC analysis, which are 2-hexanone and 6-Oxo heptanoic acid analyzed by GC-Mass. In a flight-tunnel bioassay, the chemicals induced the approach behavior of females, but the turning or jumping behavior was not observed. In the field trials, however, the chemicals did not show any attraction of B. depressa. Candidate attractants including methyl eugenol, cue-lure, protein sources, pumpkin juice, and pumpkin powder were tested. Unexpectedly, B. depressa showed no response to methyl eugenol and cue-lure, two common attractants in other fruit fly species, in the field and laboratory. Instead, the McPhail trap baited with protein was the most attractive, followed by brewer hydrolysate, protein hydrolysate + pumpkin powder, and pumpkin juice in the attraction ranking. The pumpkin powder alone did not attract the fruit fly. The crude extracts of pumpkin induced behavioral responses (attraction and oviposition) of B. depressa. Some of volatile compounds from pumpkin fruits elicited significant EAG response to female antennae. (R)-(+)-Limonen, Nonyl aldehyde, P-Cymene, Octyl aldehyde, 2-Ethyltoluolen, 3-Ethytoluol, and Cumene showed slight attraction of B. depressa adults. However, no attraction was observed in the field.

Keywords:*Bactrocera depressa, reproductive behavior, cuelure, methyl eugenol*

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PS3M422

Ecology

P3

Identification of the Trail-Following Pheromone of the Pest Termite Amitermes evuncifer (Isoptera: Termitidae)

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Amitermes evuncifer (Termitidae, Termitinae) is a pest termite in several countries of Africa, damaging crops and buildings. The trail-following pheromone of this termite was isolated from worker sternal glands and identified by GC-MS after solid phase microextraction (SPME). The pheromone is composed of (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol (dodecatrienol) and neocembrene acting in synergy, neocembrene being 5-10 times more abundant than dodecatrienol. Dodecatrienol alone is able to elicit trail-following in workers, whereas neocembrene alone is inactive. However, the association of both components induces a significantly higher trail-following activity. Multicomponent trail-following pheromones were only known, until now, in the Termitidae Nasutitermitinae, and in the Rhinotermitidae Prorhinotermes.

Keywords:*dodecatrienol, neocembrene, multicomponent, SPME, GC-MS, termites*

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PS3M423

Ecology

P3

Prediction of distribution and abundance of ants based on climate change of A1B climate scenario

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Distribution and abundance of ants are highly dependent on temperature. Therefore, the change of ant distribution due to global warming can be predicted using a temperature-based model. In order to develop the model, we surveyed ants at the 366 sampling sites of forests in the whole region of South Korea using pitfall traps from 2006 to 2009. In addition, we compared abundance of ants in the Gwangneung forest between 1993 and 2009 in order to test influence of global warming on local ant community. Of 58 ant species collected, 20 common species which occurred at more than 10% of sampling sites were analyzed on the relationship between abundance and temperature, and 16 species were found to have high relationship. The temperature-dependent species were considered as climate indicators, and their abundance and distribution in 2011, 2020, 2060, and 2090 were estimated on A1B climate scenario. The prediction shows that 11 species will decrease as the globe warms, but 5 species will increase. In the Gwanneung forest, Myrmica kotokui Forel occurred in 1993 but disappeared in 2009. This species is estimated to be most vulnerable to global warming on the model. This species is most abundant in the highlands in South Korea in 2011, but is expected to disappear almost in 2090.

Keywords:*Temperature, Forest, Climate indicator, Myrmica kotokui Forel*

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Ecology

P3

Local change of butterfly species in response to global warming and reforestation

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It is expected that the successful nationwide reforestation and global warming will greatly change the butterfly fauna in South Korea. We compared current data (2002~2007) regarding the abundance and presence of butterfly species at 2 sites in the central portion of the Korean Peninsula with similar data from the late 1950s and early 1970s for the same sites. The expected changes were documented by an abundance change of butterflies at the 2 study sites in the previous study. Using the same data, the most greatly changed species and the change of species presence were analyzed. Population changes of 99 butterfly species which occurred at both sites were significantly correlated between the 2 sites. The greatly increased species included 3 Southern (S) species and one Northern (N) species. However, the species showing great decline included 5 N species and no S species. This change coincided with the expectation of northward movement of butterfly species due to global warming. The current status of the greatly changed species is discussed along with other studies. The binary data (presence/absence) in the present study support the expected changes of butterfly species based on global warming and reforestation. The interactive effect of 2 environmental changes was also recognized, as well as the change of abundance in the previous study.

Keywords:*Temperature, Forest, Grassland, Species change, Habitat change, Butterfly, Abundance*

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Ecology

P3

Locality records of potato tuber moth, *Phthorimaea operculella* (Zeller), distributed in the Korean Peninsula

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Potato tuber moth (PTM), Phthorimaea operculella (Zeller), is a cosmopolitan pest, especially in warm temperature and tropical regions where host plants, mostly belong to family Solanacea, are cultivating. In recent years, the species has been inadvertently occurred in the potato fields of middle areas of Korea. To determine the distribution of PTM, we carried out an extensive survey using sex pheromone traps at 38 sites across the nation during 2009~2011. Delta-typed traps were baited with rubber septa impregnated with commercially available PTM sex pheromone. In each area surveyed, a potato field approximately 0.1~3 ha in size was selected and 2 traps were randomly placed in that field 1 m above ground level. As a result, we confirmed that the range of PTM extended from southern coastal regions to the central regions of the Korean Peninsula. The northern limit of PTM distribution expanded to northern Gyeonggi (Yeoncheon, Gangwha, Gimpo, Paju) and middle Gangwon regions (Goseong, Inje, Pyeongchang) from Gyeongbuk and Chungbuk regions reported first by Choi & Park (1980). Based on these data, we investigated why drastic going northward of PTM happened in Korea. Here is one presumption that a sudden rise in average temperature has occurred during 30 years, from 6.34℃ of 1973 to 7.62℃ of 2007, in the Korean Peninsula.

Keywords:*Phthorimaea operculella, Solanum tuberosum, distribution, Korea*

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Ecology

P3

Phylogenetic diversity of lignocellulosic biomass-degrading bacteria in the digestive tracks of a mole cricket (*Gryllotalpa orientalis*) and an Earthworm (*Eisenia fetida*)

Do Young Kim[1], Hyo Jeong Kim[2], Hyangmi Kim[3], Doo-Sang Park[4], Han-Young Cho[5], Yi-Joon Kim[6], Dong-Ha Shin[7], Kyung Sook Bae[8], Kwang-Hee Son[9], Ho-Yong Park[10]

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Phylogenetic diversity of lignocellulosic biomass-degrading bacteria in the digestive tracks of a mole cricket (Gryllotalpa orientalis) and an earthworm (Eisenia fetida) were investigated. Of the 86 different heterotrophic bacteria isolated from the digestive track of E. fetida, 29 bacterial strains in this study were identified as lignocellulosic biomass degraders capable of hydrolyzing carboxymethyl cellulose, xylans, or mannans. Phylogenetic analysis showed that these fibrolytic bacteria were affiliated with the Gram-positive phyla of Firmicutes and Actinobacteria. Similarly, 32 morphologically different exo-symbiotic heterotrophs were selectively isolated from the digestive track of G. orientalis. However, only 13 bacteria exhibited hydrolytic activity towards the aforementiond polysaccharide substrates. These bacterial isolates were evaluated to be phylogenetically classified into the Gram-positive phyla of Actinobacteria and Firmicutes and the Gram-negative phylum of Proteobacteria. Based on these results, it is proposed that similar to the termite digestome, the digestomes of G. orientalis and E. fetida can be also very useful as sources of diverse cellulolytic and hemicellulolytic intestinal bacteria, which are expected to possess novel genes encoding particular glycoside hydrolase (GH) enzymes.

Keywords:*diversity, lignocellulosic biomass, Eisenia fetida, Gryllotalpa orientalis, digestive track*

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Ecology

P3

Study of preservation and proliferation of the endangered species (*Argynnis nerippe*) in western islands of South Korea

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In Guleopdo, western islands of South Korea, we found an endangered species Argynnis nerippe. Guleopdo has widely grassland for Argynnis nerippe as a habitat, which can provide enough resources for them to live (Kim, Lee & Kwon 2011). In the past, grassland in Guleopdo has been used for feeding livestock, such as cows and black goats. Then, here is changed grassland from peanut farm about 80 years ago. In order to know the differences between Guleopdo and the nearby islands, we compare the fauna of the 7 islands in western of South Korea around the Guleopdo. During June to July, 2011, we visited 7 islands by 4 times field trips. We swept in day time and used light trap in night time. As a result, We found 10 orders, 50 families, 160 species in Guleopdo; 6 orders, 44 families, 141 species in Baegado; 5 orders, 11 families, 13 species, in Sungapdo; 6 orders, 15 families, 19 species in Jido; 8 orders, 19 families, 21 species in Uldo. Comparing with each islands, Baegado has the most approximate result with Guleopodo. The richness index of Guleopdo is 0.6078, and the Baegado is 6.458. The biodiversitiy index Guleopdo is 2.98, Baegado is 3.019. Now Guleopdo can afford to support A. nerippe. But, if natural disaster and ecological succession destroy grassland, the species A. nerippe will disappear in Guleopdo. So making Baegado similar with Guleopdo, we can spread and protect that species better.

Keywords:*Argynnis nerippe, endangered spacies, Guleopdo, insect, Preservation*

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Ecology

P3

Host preference of *Plutella xylostella* (Lepidoptera: Yponomeutidae) to cruciferous vegetables

Eun-jung Han[1], Jae-Pil Choi[2], Min-Ho Lee[3], Jong-ho Park[4], Yong-Ki Kim[5], Seong-Jun Hong[6], Chang-Ki Shim[7], Min-Jeong Kim[8], Hyeong-Jin Jee[9]

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The organic cultivation area has increased rapidly during the last decade in republic of Korea. Despite the outer growth of organic agriculture, management of insect pests relies on organic materials such as insecticidal plant extracts and commercial Bacillus thurigiensis, because there is no enough information about ecological pest control. In this study, we estimated preference of diamond back moth (Plutella xylostella) to 13 cruciferous vegetables in the laboratory and in glasshouse to select trap crops as a component of 'push-pull strategy' for chinese cabbage cultivation. Cruciferous vegetables tested were pak-choi, dacheongchae, two kinds of red cabbages, head lettuce, turnip, rape, kale, green leaf mustard, red leaf mustard, broccoli, red mustard and Chinese cabbage. Results from Y-tube olfactometer studies through comparing orientation of P. xylostella between treatment of each cruciferous crop and blank treatment indicated that P. xylostella was attracted by all of the cruciferous vegetables. However, in the experiment comparing attractiveness of each cruciferous vegetable to Chinese cabbage, rape attracted P. xylostella more than chinese cabbage. Oviposition preference tests conducted in a cage (60cm×60cm×60cm) showed that P. xylostella preferred to lay eggs on turnip, rape, red mustard, green leaf mustard and red leaf mustard compared to chinese cabbage. These results suggest that rape be a promising trap crop for Chinese cabbage cultivation.

Keywords:*Plutella xyllostella, cruciferous vegetable, trap crop*

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Ecology

P3

Geographical distribution of *Ixodid* ticks (Acari: Ixodidae) in Korea

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The geographical distribution and ecological preference of ixodid ticks in Korea were surveyed at 22 regions from April to September 2011. We collected 15,083 ixodid ticks with 3 genera, 7 species, using dry ice bait trap and flagging. Among them, Genus Haemaphysalis, Ixodes and Amblyomma accounted for 15,035 (99.7%), 47 (0.3%) and 1 (<0.0%). Respectively, Genus Haemaphysalis consisted of 4 species, H. longicornis (92.6%), H. flava (7.1%), H. formosensis and H. hystricis (<0.1%). Genus Ixodes consisted of 2 species, I. nippoenesis (0.2%) and I. persulcatus (0.1%). Genus Amblyomma was only A. testudinarium. H. longicornis was the most highly collected more than 52.2% in the whole region, except Hamyang-gun of 19.0%. And H. flava was collected less than 44.8% in the whole region, except Hamyang-gun of 78.6%. I. nipponensis was infrequently collected in 11 regions, and I. persulcatus was only collected in Pyeongchang-gun. The grassland site was the most highly infested site (42.1%). The collection sites for ticks consisted of coniferous forest, broad-leaved forest, mixed stand forest, grassland, bamboo grove and other sites.

Keywords:*Ixodid ticks, geographical distribution, Haemaphysalis, Ixodes, dry ice bait trap*

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Ecology

P3

Interaction between herbivores and leaf quality at rural landscape of Southwestern Korea.

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Insect and plant interaction emerges as consequence of evolution through complex physical, chemical, and environmental factors. The interaction adapts itself in different ways to the changing condition. The present study was aimed at investigating the interaction between herbivore and its food plant qualities. We collected lepidopteran larvae using beating method from May to September at a rural landscape. Two dominant food plants, Eurya japonica and Quercus serrata were chosen and nitrogen, carbon and water content of each plant were measured. We found that temporal change of species richness was related with carbon and nitrogen content (R_{adj}=0.749, $F_{1,10}$=11.044,<0.01). However, the larval populations on these two food plants were not related with plant chemical qualities. In addition we collected weather date such as mean temperature, relative humidity, quantity of solar radiation, mean wind speed, maximum instantaneous wind speed and rainfall. Temporal change of species diversity was only related with the maximum instantaneous wind speed (R_{adj}=0.501, $F_{1,10}$=11.044, <0.01). Consequently, an understanding of the interaction between herbivore and food plant should be based on the interaction of Lepidopteran larvae and leaf qualities of their food plant and its environmental factors.

Keywords:*Interaction, Herbivores, Leaf quality, Environmental factor.*

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Ecology

P3

Distribution and habitat of Korean *Osmia* spp. in apple orchard of Korea

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We investigated the distribution and habitat characterization of Korean Osmia spp. in apple orchard of 32 localities during 2005 to 2008. A total of 6,737 Osmia spp. were collected, consisting of six species, which was Osmia cornifrons, O. pedicornis, O. taurus, O. benefica, O. satoi and O. jacoti. The rate of bee nested was 11.7%. Among collected 32 localities, Choongju showed the most collecting rate among other localities, which was 22.4% and 17.5% for Yeongju and 14.9% for Geochang. In case of collected six Osmia spp, O. cornifrons was 60.2%, which was the dominant species and O. pedicornis was 20.4% and O. taurus was 10.8%, respectively. In case of sex ratio, O. cornifrons was 1 : 2.7 and 1 : 5.6 for O. pedicornis and 1 : 2.3 for O. taurus. Osmia spp. was more collected at habitat conditions within apple orchard with plentiful pollens place and abundant water.

Keywords:*Osmia spp., Distribution, Habitat, Apple*

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Ecology

P3

The genetic relatedness assay through the nuclear gene analysis in *Vollenhovia emeryi* (Hymenoptera: Myrmicinae)

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Vollenhovia emeryi ant is distinguished by its wing morphology; short winged (SW) and long winged (LW). Its reproduction shows a bizarre genetic caste system distinct from other social hymenopteran insects. Unfertilized eggs undergo genome duplication and develop into clonal gynes. Fertilized eggs develop either into workers or males. The fate of the fertilized eggs is determined whether maternal genome loss (MGL) takes place after fertilization. Eggs with MGL become haploid males with only paternal half of the genome. Without MGL, the eggs become workers with maternal and paternal half of the genome. In this research, we analysed 5 nuclear genes of SW and LW individual ants. Among them, two genes from an SW male are identical to those of LW, and one gene from the SW male seems a variant of LW. The result indicates that SW males are derived from LW colonies. From the genetic relatedness point of view individuals in the same castes are genetically identical. On the other hand, between workers and two reproductives, the relatedness is asymmetrical and there is even no gene sharing between gynes and males. The conventional genetic relatedness by Hamilton is revised under this condition.

Keywords:*genetic caste system, wing morphology, genetic relatedness*

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Ecology

P3

The mitochondrial genetic population structure of the ant species, *Vollenhovia emeryi* (Hymenoptera: Myrmicinae) in Korea

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The ant species, Vollenhovia emeryi, is distributed in Far East. The species can be divided into two major groups by their wing morphology of reproductives: short-winged and long-winged. A nationwide survey of the species was conducted for analyzing the mitochondrial haplotype diversity and genetic population structure. We collected 91 samples from 40 locations. A total of the 1239 bp partial COI (cytochrome C oxidase 1) region was used for the analyses. We found the total of 21 haplotypes. The mitochondrial haplotypes may correspond to the wing morphology. The genetic population structure examined potential geographic barriers of gene flow such as distance, mountains, rivers and plains which are non-mountain areas to prevent dispersal through mountain range. The result implied that no barriers considered in this study affected differently gene flow. Therefore, the behavioral characteristics of the ant may be the causal constraint of its genetic exchange.

Keywords:*Vollenhovia emeryi, wing morphology, population structure, mt-COI haplotype diversity, gene flow bar*

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Ecology

P3

Anti-allergic effects of venom from honeybees (*Apis mellifera* L.) on production of nitric oxide and cytokines in THP-1 Cell line and ige level and the scratching behavior in NC/Nga mice

Hyun Bae Kang[1], Hae Jin Lee[2], Sang Jin Jeon[3], Sang Kyun Koh[4], Ji-Sook Lee[5], Chi-Young Yun[6]

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Bee venom (BV), as traditional oriental medicines, has been well known the pain reliever and anti-inflammatory agent for a long time, and shown anti-arthritic and anti-carcinogenic effects. In this study, we investigated the effect of bee venom on the THP-1 cell stimulated by house dust mites (HDMs), which mediate the activation of T cell and monocyte, and induce the elevation of serum IgE levels in atopic dermatitis (AD) patients, for the elucidation of anti-allergic activity. THP-1 cells activated with HDMs were treated with different doses of BV and then the supernatant was analyzed for the production of NO and cytokines using Griess reagent and ELISA, respectively. RNA levels in the THP-1 cells were measured by a reverse transcriptase-polymerase chain reaction. The results indicated that BV suppressed HDMs-induced NO and inducible NO synthesis expression in a dose-dependent manner, and also represented that BV exerted anti-allergic effects by suppressing the transcription of cytokines, such as IL-6, IL-8, IL-13, TNF-α, ICAM-1 and MCP-1. We also examined the dorsal skin administration of BV to NC/Nga mice treated by 2,4-Dinitrochlorobenzene (DNCB) was found to inhibit increase of the ear thickness and the skin lesions. In the evaluation of the scratching behavior, it decreased in 15-20%. The serum IgE production of the mice was significantly inhibited by BV treatment. These results suggest that BV has anti-allergic activities, and thus can be useful in inhibiting the production of some cytokines in AD. Further studies involve the analysis of the individual components of BV and their pharmacological aspects.

Keywords:*Bee venom, House dust mite (HDM), Atopic dermatitis (AD), Nitric oxide (NO), Cytokines, 2,4-Dinitrochlorobenzene (DNCB), Nc, Nga mice*

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PS3M435

Ecology

P3

Occurrence patterns of three major fruit moths, *Grapholita molesta*, *Grapholita dimorpha* and *Carposina sasakii*, with sex pheromone in plum orchard

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Fruit borers such as Grapholita molesta, Grapholita dimorpha and Carposina. sasakii are major pests of plum in Korea. Population densities and seasonal occurrence were monitored using the synthetic sex pheromone trap in Uisung and Gyoungsan, two important plum growing area in Korea. In 2010 and 2011, overwintering adults of G. molesta were caught from mid April with the peak of late April, and then undergone 3 more generations in Gyoungsan. Grapholita dimorpha appeared from late April with 3 peaks afterward until late September. Both Grapholita species occurred a few days later in Uiseong where the latitude is 1? high. There was no difference between the season of C. sasakii between Gyoungsan and Uiseong with the same patterns of 2 or 3 peaks. Population size was in order of G. molesta, G. dimorpha and C. sasakii with 63:16:20 and 47:35:18 in Gyoungsan and 51:18:31 and 46:13:36 in Uiseong, in 2010 and 2011 respectively. There was no difference of catches of Grapholita species in the trap installed inside and outside of the orchards.

Keywords:*trap, monitoring, fruit borer, plum, dimorpha, occurrence pattern*

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PS3M436

Ecology

P3

Diversity of gamasid mites (Acari: Mesostigmata, Gamasida) in different agroecosystems

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Gamasid mites are important predators in soil ecosystem including agricultural lands. We compared the gamasid mite diversity in different agricultural systems; pine forest as a natural ecosystem, apple orchards as a perennial crop, potato and Chinese cabbage fields as an annual crop. Diversity and abundance were highest in the pine forest followed by apple orchards and then potato and Chinese cabbage fields. In the pine forest, more species and individuals were collected from the primary forest compared to the secondary forest. In apple orchards, more species were found from the organically managed orchards than from the conventionally managed ones, but the abundance patterns were in reverse. Annual crop system harbors least diversity and abundance of soil gamasid mites indicating that the disturbance and input of organic materials influence the gamasid assemblage. Dominant species were Asca nubs, Parasitidae sp. 2, 3, 4, Gamasholaspis browning, G. communis, Holaspina alstoni, H. dentatus, H. sp. 1, Veigaia ashizuriensis, V. ochraceus, V. tibbetis in the pine forests, Eugamasus fujisanus, Holaspina ochraceus, Leioseius insignis, Uropoda hikkaidoensis, V. ashizuriensis, V. uenoi in the apple orchards, and Leioseius sp. 1 in the annual crops.

Keywords:*Gamasid, Predator, Agroecosystem, Disturbance, Annual crop, Perennial crop, Organic input*

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Ecology

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Comparison of oribatid mite communities between the pine forest and apple orchards

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Community structures of oribatid mites in the pine forest and apple orchards were compared as an example of natural and disturbed habitat of oribatid mites. Diversity and abundance were higher in the pine forest than the apple orchards. In pine forest, primary forest harbored more diversity but less abundance when comparing to the secondary forest. However, the diversity was higher in conventionally managed apple orchards than organically managed ones but the abundance was not different. Common dominant species in pine forest were Cultroribula lata, Ceratozetes japonicas while Epidamaeus khustaiensis, Eremobelba japonica and Eremaeus tenuisetiger were dominant in primary and Oppiella nova, Scheloribates pallidulus were in secondary. In apple orchards, Pergalumna duplicata nipponica, Scheloribates latipes were the common dominant species while Hypochthonius luteus was dominant in organic apple orchard and Pergalumna altera, Scheloribates corpusculum, Punctoribates sphaericus were dominant in conventional orchard. Further study on the functional aspects and association with other soil biotic and abiotic factors are demanding.

Keywords:*Pine forest, Primary forest, Secondary forest, Apple, Disturbance*

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Ecology

P3

Comparison analysis of acoustic signals to *Liriomyza trifolii* (Diptera: Agromizidae) with the EAG signals

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We investigated an antenna response of Liriomyza trifolii (Diptera: Agromizidae) to acoustic signals by using the EAG (Electroantennogram) technique. Originally, EAG have been used to observation of neural transferring of the olfactory response in antenna of insect. It seems quite probable, because the auditory organ known as Johnston's organ in fly was located in antenna. In this study, we tried to utilize the EAG for comparison the relationship with acoustic signals and antennal response. Pure tone sounds (sine wave), which were varied from 20 to 1,000 Hz according to different sound pressures (50 ~ 100 dB), were exposed to excised head of test specimens. Neural response in antenna was strongly appeared against low level frequency and high level sound pressures. In conclusion, EAG signals would be useful for detecting the antenna response to acoustic signals.

Keywords:*Lirimyza trifolii, Insect sound, EAG, Response behavior, Pest management*

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Ecology

P3

Genetic variation in tendency to diapause in spider mite *Tetranychus puerariicola*

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12 iso-female lines were established from a population of the adult-diapausing spider mite, Tetranychus puerariicola. The diapause incidences of each line were investigated for 18, 19, and 20°C and LD 10:14 h conditions. There was significant genetic variation in the each temperature conditions. Genetic correlation between temperatures was about 0.7 in either combination.

Keywords:*diapause, genetic variation, genetic correlation, life-history traits*

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Ecology

P3

Life table-based computer simulation of population dynamic of *Spodoptera litura* (F.) fed on groundnut (Arachis hypogaea) leaves under different condition

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Spodoptera litura (F.) is the most destructive leaf-defoliating pest of peanuts (Arachis hypogaea) in Taiwan. To accurately simulate the population dynamics of S. litura, life table data, using the age-stage, two-sex life table, were collected at constant temperature 25ºC in the laboratory, and outdoors under field conditions in the spring and fall of 2010. Results showed that there were significant differences in developmental times, survival rate, fecundity, and hatch rate under different conditions. The shortest immature stage occurred in fall (19.2 d), while the longest was in spring (28.4 d). At 25℃, both male and female adults lived longer than adults under field conditions. The mean generation time was the shortest in fall (34.5 d). The preadult survival rates in spring (39.9%) and fall (53.3%) were lower than the rate observed at 25℃ (84.6%). The mean fecundity of S. litura was 3548, 3452, and 3072, at 25℃, in spring, and fall, respectively. The hatch rates were 64.2, 72.2, and 26.0%, respectively. At 25°C, the intrinsic rate of increase (0.1838 d-1), finite rate of increase (1.2017 d-1), and net reproductive rate (963.2 offspring) were the highest among all treatments. Population parameters showed high variability in spring and fall. Because both hatch and survival rates in the field were affected by various unregulated environmental factors, they were lower than those obtained at constant temperature. Our results demonstrate that computer simulation based on life tables collected under both laboratory and field conditions is an important tool for prediction pest emergence.

Keywords:*Spodoptera litura, Arachis hypogaea, life table, fecundity, sumulation*

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Ecology

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Multiple generation effects of high temperature on the development and fecundity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B

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Insects are ectotherms and their ability to resist temperature stress is limited. The immediate effects of sub-lethal heat stress on insects are well documented, but longer-term effects of such stresses are rarely reported. We studied sub-lethal high temperature effects on the survival, development and reproduction of invasive whitefly Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) biotype B over five successive generations. Both temperature and generation significantly affected the fitness of the whitefly. Among the tested temperatures of 27°C, 31°C, 35°C and 37°C (± 1°C), the most favorable for development and reproduction were 27°C and 31°C. At 27°C, survival, development and fecundity were all stable over these five generations. At 31°C, egg hatching rates decreased in the third to fifth generations, female fecundities decreased in the fourth and fifth generations, and female percentage and adult longevity fluctuated among the generations. At 35°C, egg hatching rate and immature survival rate decreased and immature development time increased with generations, female fecundities decreased in the fourth and fifth generations, and female percentage and longevity fluctuated among the generations. Female fecundity at 37°C was less than 10% of that at 27°C or 31°C and the whitefly culture at 37°C lasted for only one generation. The lower threshold of lethal high temperature of B. tabaci biotype B was higher than 37°C, and the whitefly population continued expanding in the five generations at 35°C. The ability of B. tabaci biotype B to survive long-term high temperature stress will play an important role in its population extension under global warming.

Keywords:*Bemisia tabaci biotype B, high temperature stress, long-term fitness effects, population expansion*

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Ecology

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Ecological niche of 16 species of bark beetles (Coleoptera: Scolytidae) within *Pinus armandi* of Huoditang located ecosystem station of Tsinling Mountains

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Bark beetles, the major pest caused a fatal damage of Pinus armandi of Huoditang Foresty Station in Tsinling Mountains, were investigated on organization level and temporal-spatial niche. The result showed that the bark beetles community was abundant, containing 16 species of them. Dendroctonus armandi, Cryphalus lipingensis, Ips mannsfeldi and I. sexdentatus were the major species. Analyzed by Levins, Shannon-Wiener and Pielou formulas, we concluded that they've appeared to vertical distribution. D. armandi was the pioneer species because of the largest number of individuals and the most broad niche breadth. Besides, Hylastes parallelus and Polygraphus polygraphus occupied within the roots and the base of trunk. Yet some Ips bark beetles were densely distributed in the top of tree trunk and branches, such as Tomicus piniperda and Cryphalus lipingensis. Other bark beetles, Hylastes parallelus, Dermestes sp., Dermestes sp., etc. were distributed with relatively narrow breadth. The statistical data reflects other 7 species had their own spatial and overlap niche within the main trunk. So all these species hold with a dynamic model of competitive equilibrium to coexist by their biological and ecological characteristics.

Keywords:*bark beetles, ecological niche, vertical distribution*

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Ecology

P3

Efficiency of ten population of *Schizaphis graminum* (Rondani) in transmission of barley yellow dwarf virus-PAV (BYDV-PAV) in Iran

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Variation in transmission of ten clones of Shizaphis graminum (Rondani) originating from different region of Iran was evaluated by transmitting an isolate of barley yellow dwarf virus-PAV (BYDV-PAV) for which this species is normally an inefficient vector. After acquiring the virus, aphids of each clone-virus isolate combination were caged on 30 seedling using 3 forth instar nymph per seedling. The inoculated and control plants were assayed by ELISA and assayed visually to determine the rate of transmission and the mean ELISA value for each treatment. A positive correlation was found between the rate of transmission and the mean absorbance value. Aphid clones differed in their efficiency of transmission of virus isolate. On the other hand, any single clone transmitted virus isolate with varying efficiencies. The aphid clone belonging virus isolate origin transmitted virus isolate at highest rate. In general Shahre kord and Shahreza clones of aphids showed highest efficiency of transmission and Delijan and Varamin clones have lowest transmission efficiency between aphid clones.

Keywords:*luteovirus, aphid clones, BYDV-PAV, transmission efficiency, Shizaphis graminum, Iran*

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Ecology

P3

Photosynthesis and sink activity of cecidomyiid galls in Litsea acuminate leaves

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Multiple changes in response to gall inducers have been found in host plant tissues. While much attention has been focused on the morphology and anatomy of insects or their induced galls, relatively little work has been done on the photosynthesis. In this study, we used pigments and soluble protein contents, gas exchange, chlorophyll fluorescence, scanning electron microscope and confocal laser scanning microscope to investigate the photosynthetic characteristics of leaf-derived cecidomyiid galls and their leaves. In contrast to the host plant leaves, herbivorous insects altered both chlorophyll biosynthetic and degradation pathways of galls on host plants. The gall tissues had lower solute protein contents than leaves. The gas exchange of gall was not found, and the stomata were also not found on the surface of galls. Furthermore, photosynthetic performance was also evaluated by measuring chlorophyll fluorescence. When compared chlorophyll fluorescence in leaves and gall, fluorescence intensity of gall tissues was lower than that of leaves. Based on our survey, we suggest that the leaf-derived cecidomyiid galls are novel sinks in Litsea acuminata leaves.

Keywords:*photosynthesis, cecidomyiid gall, chlorophyll fluorescence, Litsea acuminata*

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Ecology

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Influence of temperature on population growth parameters of *Agonosena bimaculata* on Khinjuk trees, *Pistacia khinjuk* and *cuptivated pistacia*

Neda Fallahnejad Mojarrad[1], Sohrab Imani[2], Mina Khajoeinejad[3], Mohamadreza Mehrnejad[4], Karim Kamali[5]

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The Khinjuk psylla , Agonoscena bimaculata Mathur was reported as a pest of the Pistacia khinjuk stocks in Khajoei mountainous area in Sirjan , siuthern part of Iran . In this research ,in fluence of temperature on population growth parameters of A. bimaculata on Khinjuk trees , P. khinjuk and cuptivated pistacia in laboratory conditions at 25 , 27.5 and 32 Cº , 55±5 % RH and photoperiod of 16:8 (L:D) hours.The lowest developmental time for psyllid eggs and nymphs was obtained at 32 ºC, 4.4 and 11.1 days respectively . The in fluence of host plants , e.g. P . vera and P. khinjuk on fecundity of this psyllid were checked . The results showed that egg laying of A. bimaculata under controlled condition is larger on P.khinjuk although the differences were not significant.

Keywords:*Temperature, Agonosena bimaculata, population growth parameter*

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Ecology

P3

Insects associated with plantations of some exotic and indigeneous tree species in Ondo state

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The study was carried out to examine the effect of plantation of different tree species on Arthropod diversity and abundance. Three plantations comprising Nauclea diderichii, Gmelina arborea and Tectona grandis within Ondo state were selected for the study. Sample plots of 50 m x 50 m were demarcated in each of the selected sites. Insects sampling and identification was carried out using sweep nets. Soil samples were also collected from each of the selected plots at 0-10cm depth and were carried to laboratory for mesofauna isolation and identification. A total of 43 insects were collected and identified, 17 insects from the 15 years Nauclea plantation, 14 from the 9 years and 12 individuals from the 5 years plantation. A mean abundance of 44 individuals of mesofauna were also encountered, 20 from the G. arborea plantation, 5 from the T. grandis plantation, and 19 from N. diderichii plantation. Shannon Weiner diversity (H') index, for the three age series 1.67, 1.63 and 1.62 for the 15, 9 and 5 years old plantation respectively. While (H') index for the G.arborea and T. grandis plantations were 1.63 and 1.68 respectively. There were significant differences in the mean mesofauna abundance ($P \leq 0.05$) from the samples obtained from different plantation. Also there was no significant ($P \geq 0.05$) difference in the insect species diversity and abundance in all the sites that was studied.

Keywords:*Mesofauna, diversity index, plantation, Shannon Weiner*

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Ecology

P3

Change of ant species composition in urban parks over time

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To compare the change of ant faunas in urban parks over time, we surveyed two parks in Fukuoka City, Japan: Momochi Chuou Kouen Park (MCP) and Minami Kouen Park (MKP), both of which were studied in 1998. The survey was conducted in 2012 using 30 min. time unit sampling with 8 replicates. This sampling method is exactly the same as the one executed in 1998. In MCP, 16 species were collected and estimated species number was 17.63 to 21.47 by Jack- Kinfe method. Compared with the data of 14 years old, the collected species number and estimated species number were almost same (17 spp. with 17.60 to 19.90 spp. in 1998). However, 4 species (Ochetellus glaber, Formica japonica, Nylanderia flavipes, Pyramica membranifera) were not found in 2012 and 3 species Temnothorax spinosior, T. congruus, Monomorium intrudens were newly recorded. This could be a turnover of species. MCP has a simple environment with open space and peripheral artificial vegetation managed regularly. The strong human disturbance may cause the extinction of local population and immigration of ant species. On the other hand, the MKP result showed 19 collected species (20 spp. in 1998) with 24.68 to 27.32 estimated species (23.50 to 27.00 spp. in 1998), where 6 species were not collected this time and 3 species were newly recorded. However, the species change in MKP cannot be confirmed, because the sampled species were too low comparing with estimated species number.

Keywords:*Species composition, urban park, turnover, human disturbance*

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Ecology

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Twig-nesting ant diversity in lowland forests of Cambodia

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Twigs are one of the important resources as nesting site for ants, but there has been poorly investigated in tropical Asia. We surveyed two lowland forests (Kampong Chnang, KC and Kampong Thom, KT) in Cambodia, both of which were dominated by evergreen tree species with deciduous tree such as Dipterocarpus spp. Samplings were conducted by collecting dead twigs on trees found within a radius of ca 2 m at each sampling point, and those on the ground found within a 1 m x 1 m quadrate. Each sampling was replicated 8 times in December 2011. In total, 24 species were collected: 12 species were collected in KC, 15 in KT. Three species, Crematogaster ferrarii (17% all colonies caught), Monomorium pharaonis (17%), Tapinoma melanocephalum (13%) were dominant in KC, whereas only one species, Philidris sp. (32%) was dominant in KT. The number of singleton species was higher in KT (12 spp.) than in KC (6 spp.). Some invasive species such as Tapinoma melanocephalum and Monomorium pharaonis were frequently collected in KC. Species of Crematogaster (Colobocrema), and Vombisidris were rare and found in KT. The total species richness of twig-nesting ants, including both arboreal and ground samples in the two forests, is roughly estimated to be around 50 species (Jackknife 2). KC is a community forest and the floors are poorly covered by vegetation, whereas KT is a regrowth forest with abundant shrubs and weeds.

Keywords:*Ants, Cambodia, diversity, twig-nesting.*

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Ecology

P3

Seasonal abundance of horse fly (Diptera: Tabanidae) in Western Anatolia

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Adult horse flies take blood meals from many mammals including human, so they have known as serious pests wherever they occur and also are known vectors of numerous disease agents. Seasonal abundance of Tabanidae species was monitored during from May to September of 2005, 2006 and 2007 years, in Western Anatolia, Turkey. Totally, 7293 specimens from 52 species belonging to nine genera were collected and identified. Seasonal activities of ten most abundant species, Dasyrhamphis umbrinus, Haematopota subcylindrica, Philipomyia aprica, Tabanus bifarius, Tabanus bromius, Tabanus lunatus, Tabanus portschinskii, Tabanus quatuornotatus, Tabanus rupium and Tabanus unifasciatus were analyzed. D. umbrinus, T. bifarius, T. quatuornotatus, T. unifasciatus reached their peak of abundance in June and others species reached their peak of abundance at July. The longest flight period from first week of May to mid-September was determined for the species Haematopota subcylindrica and Tabanus bromius. Moreover, Haematopota subcylindrica was the most abundant with 23.86% and followed by Tabanus bromius with 20.65%. The population peak of species, and also vector potential, for each year can be affected from climatic conditions. Nevertheless, results suggest that the time between from the mid-June to the third week of July can be considered as the potentially higher-risk period of mechanical transmission of pathogens by tabanids for the study area.

Keywords:*Tabanidae, Seasonality, Flight Period, Western Anatolia, Turkey*

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Ecology

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Detection of extra host plants for some agricultural insect pests in Sudan

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Numerous phytophagous insects were reported to attack variable host plants (cultivated and wild) since earlier times in Sudan. But, lacking of regular inventories and updates pertained to such plant-insect interactions has led to conceal the real situation, which represented by the appearance of new pests and/or new hosts. Therefore, a part from the findings obtained as a result of sporadic field surveys carried out during the last few years, was the discovery of new hosts for some known agricultural pests in the country. Hence, an attempt was made in this paper to lists such new host plants for seven important pest species. These pests included Bemisia tabaci (Genn.), Aphis nerii Boy., Liriomyza spp., Urentius hystricellus (Richter), Urentius euonymus Distant, Galeatus scrophicus Saunders and Pachnoda interrupta Oliver. More than twenty new records were listed, included 2, 2, 5, 1, 2, 6 and 3 hosts for the previous pests, respectively. However, the accelerated trade movements (nationally and internationally), besides the modern trends in agricultural systems seem to contribute in distribution of new pest and plant species and to impact the natural ecological habitats which ultimately interfere with insect-plant relationships. Consequently, the paper emphasizes the importance of continuing careful studies on these aspects so as to revise the older information and to cope up with any possible future changes.

Keywords:*New hosts, insect pests, natural habitats, Sudan.*

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Ecology

P3

Utilization of *Anthophora plumipes* (Hymenoptera, Apidae) for pollinating various flowering plants inside the closed conditions

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Anthophora plumipes is a vernal univoltine solitary bee, which is known to be a good pollinator of some plants. To further demonstrate its utilization under closed conditions, foraging and nesting activities were examined by rearing this bee in a greenhouse at Matsue, Shimane Prefecture, Japan (35º29'N, elevation 170 m). Major floral resources provided for foraging were Phacelia tanacetifolia, Borago officinalis, Centaurea cyanus and Solanum lycopersicum. Bees were attracted to artificially made soil blocks quickly after releasing into the greenhouse. Male bees also started chasing females within 12 hours of release. Bees started foraging before 6:00 in the morning and continued until 19:00 even at a low temperature of 9.5ºC to a high of 36.4ºC, showing a high stability over a wide range of temperature. Bees started collecting pollen after 4 days of liberation. The way of collecting pollen was different among the flower types. One female bee made 1-3 nests of single series and/or multi-series type(s). Single series nests had 1-4 cells, and there were 4-8 cells in multi-series nests. In a nest, initial cells were nearly horizontal, followed by brood cells falling downwards. A good brood production (5.13 per female), long active period (more than 35 days) and higher survival rate (at least 75%) of this bee showed high adaptability for foraging and nesting in the closed condition. Their foraging behavior can be judged to be highly efficient, thus can be used as a pollinator of various crops inside the greenhouse in poor facilities.

Keywords:*Anthophora plumipes, greenhouse, foraging, nesting*

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Ecology

P3

Origins of virulence: Genetic mechanisms of virulence inheretance in soybean aphid (*Aphis glycines*) biotypes.

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An invasive species, the soybean aphid (Aphis glycines) was introduced to North America in 2000 and quickly established itself as the premier insect pest of soybean on the continent. Host plant resistant (HPR) soybean was bred shortly after the invasion to provide plant based resistance against the aphid, with plant defense stemming from the Rag1 (Resistance to Aphis glycines) gene. While Rag1 soybean was initially effective, an aphid biotype virulent to the plants was discovered prior to commercial utilization. The Rag1 virulent biotype is referred to as biotype 2, while aphids remaining susceptible to the effect of Rag1 are called biotype 1. Using 20 single nucleotide polymorphism markers we compared genetic diversity and distance between concurrently collected and geographically identical populations of biotype 1 and 2 soybean aphid. Populations were collected over a micro and macro-geographic scale across northern Ohio, USA. Preliminary results indicate geographic distance between populations to be more explanatory of genetic relatedness than biotype affiliation, suggesting virulence is controlled through phenotypic plasticity rather than through specific gene inheritance. A plastic virulence phenotype has considerable implications for the implementation of HPR soybean in industrial agricultural systems, and may render the management of aphid virulence more difficult.

Keywords:*Aphis glycines, biotype*

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PS3M453
Ecology
P3

Information theory and insect ecology: Read before you apply

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Despite various studies emphasizing problems with the interpretation of diversity indices, these indices are being increasingly applied. Probably because of the high species richness of insects, diversity indices are still very popular in entomology. Here, we briefly review diversity indices with emphasis on the Shannon?Wiener index, which is the most popular index and which is based on information entropy measurement. We indicate that the importance of the diversity indices is frequently overestimated and that the indices are often calculated without reasonable interpretation. Detailed analysis of published papers shows that although modern numerical ecology and insect ecology abandoned this trend during the last decade, diversity indices are increasingly used in applied ecological sciences (forestry entomology, agriculture entomology, etc.) We urge that researchers, and especially those working in areas concerned with economic impact, not calculate diversity indices without fully understanding their limitations.
This study was funded by the Institute of Environmental Technologies project, reg. no. CZ.1.05/2.1.00/03.0100, supported by the Research and Development for Innovations Operational Programme and CZ.1.07/2.2.00/28.0149.

***Keywords**:numerical ecology, diversity indices, information entropy*

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Ecology

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Faunistic data and global warming: Misleading approach or helpful information?

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Various authors have recently provided evidence of global warming based on historical data of species distribution and abundance. This approach can be problematic for several reasons. First, causality is unclear. Without experimental design, relationships between climate change and the distribution and abundance of species must be interpreted with great caution. Second, trends in abundance or distribution can be artifacts of inappropriate methodology and especially of unequal sampling effort, which is a major difficulty when faunistic data are analyzed. Here, we use two model insect species as examples of vulnerable organisms with varying trends in population parameters during last years ("relative to climate change"). We demonstrate how unequal sampling effort affects the trends, and we propose some methods for reducing the effect of unequal sampling effort and to thereby obtain more useful outcomes. This study was funded by Institute of Environmental Technologies project, reg. no. CZ.1.05/2.1.00/03.0100, supported by the Research and Development for Innovations Operational Programme and CZ.1.07/2.2.00/28.0149.

Keywords:*numerical ecology, Odonata, Orthoptera*

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Ecology

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Study of wintering stock of cabbage flies

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To study the over wintering stock of cabbage flies in the autumn after harvesting cabbage, conducted stratified analyzes of the soil. The size of soil samples was 25-25-30 cm in the laboratory the collected puparia were separated by type, in the spring and summer cabbage flies. Puparium washed in water and after drying weighed on torsion scales. Analysis of the weight characteristics puparia of cabbage flies showed that the spring cabbage fly puparia formed in the range of OT3-25 mg/instance. Most of the puparia refers to the weight category of 11-15 mg /instance. Formed a flying cabbage root fly pupae in the range ot5-30 mg /instance, with most of the puparia belongs to the weight category of 11 to 25 mg /instance. Found that the varietal characteristics of cabbage plants can significantly affect the weight of puparia. Analysis of the characteristics of puparia weight spring and summer cabbage flies in different soil horizons showed that the greater weight of puparia, the deeper in the soil they are located. Found that the greater weight of puparia, the greater the likelihood of departure from a female pests, and vice versa, from lightweight puparia, usually predominantly male. Observations of the flight dynamics of cabbage flies in the laboratory showed that at the beginning of summer in the pest population is dominated by males, in the future there was almost certain departure of females.

Keywords:*cabbage flies,puparia,wintering stock,*

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Ecology

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Foraging cycle of Asiatic honeybee *Apis cerana* in relation to qualitative and quantitative variations in floral rewards of some plants

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Studies were conducted to determine the foraging behaviour of honeybee Apis cerana in relation to variation in nectar characteristics in nectar yield plants viz. Moringa oleifera, Millettia pequensis, Pongamia pinnata and Mangifera indica. All the four plants yielded nectar continuously throughout the day (06:00 to 18.00 hrs). Significant variations occurred in the nectar production pattern of all these plants with peak periods during late morning and noon hours. The nectar production ranged between 0.039+ 0.0042 µl and 0.036+ 0.0042 µl respectively. Similarly, nectar sugar concentration varied significantly between 17.35+ 0.41 and 61.40+ 1.49%. Variations in nectar volume and concentration were found to be significantly influenced by changes in ambient temperature and humidity. Bee visitation was significantly correlated with nectar sugar concentration and nectar secretation pattern. Interestingly, foragers collecting nectar were more predominant during afternoon whereas pollen collectors outnumbered during early morning hours. The rates of foraging and visitation activities of honeybees species indicated the total absence of inter-species competition which is an important attribute for existence of all honeybee species under particular set of conditions The present paper attempts to gain insight into different behavioral pattern of honeybee Apis cerana in relation to floral rewards of flowers and their pattern of presentation in relation to ecological conditions.

Keywords:*Forahing cycle, Apis cerana, floral rewards*

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Ecology

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Pyrosequencing reveals dominance of *Arsenophonus, Buchnera*, and *Wolbachia* in bacterial communities of an invasive aphid

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The soybean aphid (Aphis glycines), a serious pest of soybean, has successfully invaded and adapted into North America, where it was introduced from Asia around year 2000. To understand the potential role of bacterial symbionts in the invasive success of A. glycines, we performed a comprehensive survey of bacteria in A. glycines using 16S rDNA pyrosequencing. Our results suggested that Arsenophonus, Buchnera, and Wolbachia were the major bacteria associated with A. glycines. A few minor genera (relative abundance between 0.1-1.0%) such as Pantoea, Bacillus, Brevundimonas, Lysobacter and Acinetobacter were also identified from pyrosequencing data. Comparison of bacterial communities associated with field-collected (FC) and laboratory-reared (LR) A. glycines samples revealed significant differences in their structure as well as composition. We identified 18 operational taxonomical units (OTUs) that resulted in differentiation of bacterial communities of FC and LR A. glycines. Selection pressures from natural enemies and environmental conditions on field A. glycines were suggested as factors behind differential bacterial communities observed in FC and LR samples. Finally, we discussed the potential roles of facultative symbionts in A. glycines in the light of aphid biology and insect-bacteria symbiotic systems.

Keywords:*Soybean aphid Pyrosequencing Arsenophonus Buchnera Wolbachia*

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Ecology

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Age-stage, two-sex life table of *Brontispa longissima* (Gestro) (Coleoptera: Hispidae) feeding on four palm plant varieties

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The coconut leaf beetle, Brontispa longissima (Gestro) (Coleoptera: Hispidae), is a leaf-feeding beetles native in southeast Asia and the Pacific, and the beetle is a high risk pest of economic importance in South China. Here, the life history of Brontispa longissima (Gestro) (Coleoptera: Hispidae), reared on leaves of coconut (Cocos nucifera Linn.), royal palm (Roystonea regia), bottle palm (Hyophore lagenicaulis) and fishtail palm (Caryota ochlandra Hance) was analyzed using age-stage, two-sex life table under laboratory conditions. Means and standard errors of population growth parameters were calculated using the jackknife method. Moreover, survival rate and fecundity data was applied to project the coconut leaf beetle population in revealing the different stage structure of populations. The intrinsic rates of increase reared on C. nucifera, R. regia, H. lagenicaulis, and C. ochlandra were 0.032, 0.031, 0.019 and 0.044 day, respectively. The B. longissima reared on C. nucifera achieved the highest net reproduction rate at 114.547 offspring pre female. The mean times of B. longissima ranged from 93.178 day(reared on C. ochlandrai) to 161.516 day (reared on H. lagenicaulis). Population projections showed that populations of B. longissima were able to grow considerably faster on C. ochlandra than on the other three host plants. Our results demonstrate that we can accurately describe the survival, development, and fecundity of B. longissima reared on different host plants using the age-stage, two-sex life table. We recommend the age-stage, two-sex life table be used in insect demographic studies.

Keywords:*Brontispa longissima, palm plant, growth and development, fecundity, life table*

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Ecology

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Two-sex life table analysis of population characteristics of Cadra cautella Walker (Lepidoptera: Pyralidae) on dry and semi-dry date palm varieties of Iran

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Life table of almond moth, Cadra cautella Walker (Lepidoptera: Pyralidae) was studied on four main dry and semi-dry date palm varieties (Deyri, Zahedi, Piarom, and Rabbi) of Iran under laboratory condition. Data were analyzed based on the age-stage, two-sex life table theory. Duration of total preadult stages was 42.54, 45.79, 51.48 and 50.41 days on Deyri, Zahedi, Piarom, and Rabbi, respectively. The highest female fecundity of C. cautella on date palm varieties was 245.29 eggs on Zahedi. The intrinsic rate of increase (r) on different varieties of date palm ranged from 0.070 d-1 (on Piarom) to 0.106 d-1 (on Deyri). The highest net reproductive rate (R0) was on Deyri (97.13 offspring) and the lowest value of this parameter was on Rabbi variety (42.16). Our results showed, the highest r, the largest fecundity and the shortest generation time of C. cautella were observed on Deyri variety. Among date palm varieties, Deyri variety is preferred by C. cautella than other varieties.

Keywords:*biology, Cadra cautella, date palm, demography, Iran, two-sex life table*

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Ecology

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Seasonal monitoring of the major okra foliage pests and their associated predators complex at El-Gorair Scheme in Merowe Locality, Northern Sudan

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Okra [Abelmoschus esculentus (L.) Moench] is one of the important vegetable crops in Northern Sudan and many other parts of the country as well. The crop is subjected to great annual losses due to several economic pests like the cotton whitefly (Bemisia tabaci) and cotton aphid (Aphis gossypii). Besides their debilitating effect as a result of sucking plant nutrients, these pests are also known to transmit serious viral diseases to the crop. Although, insecticide application is the sole measure of control practiced in many areas, it is not widely used in this region. Therefore, it was aimed by this research to monitor the occurrence of pests and predators complex associated with okra foliage at El-Gorair scheme in Northern Sudan, as a step forward to evaluate the potential natural pest control in that area. Hence, the prevalent pests and predators were surveyed on okra fields, and their seasonal trends were studied. More than ten predatory species including members of coccinellids, chrysopids, syrphids, mantids and spiders were detected at variable levels attacking mainly B. tabaci and A. gossypii besides other soft insects. Chrisopids and spiders were the dominant predators in autumn and summer seasons, whereas syrphids, chrysopids and some coccinellid species were the abundant groups during winter. However, among the recorded species Chrysoperla carnea seemed to be the most important one in okra crop all the year round. Such rich fauna of natural enemies should be emphasized to encourage ecological control of vegetable pests and to minimize the need for chemical control.

Keywords:*Okra pests, predators, seasonal abundance, Chrysoperla carnea, Northern Sudan.*

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Ecology

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Faunestic survey on the pests of poplar trees species and their distribution

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Area increase of poplar plantations prepares suitable locations for pests' activity. Recognizing the poplar fauna is the first factor in the current study and is useful in integrated pest management. This survey was carried out from 2008 to 2011, in the plantations and nurseries (with the native and exotic poplar species and clones) of West Azerbaijan. The poplar pests were collected by installing light trap, using Steiner trap, sweeping net, direct capture, hand picking, net clothes around infested tree trunks, and rearing larvae of some pests in laboratory, on Populus species such as: Populus nigra, P. deltoides, P. alba, and P. euramericana. At last, 19 species were collected and identified which were as follows: Amorpha populi L., Lymanteria dispar L., Gypsonoma aceriana Froel. Polyphylla olivieri Castelnau, Paranthrene tabaniformis Rott., Chaitophorus populeti Panzer., Cerura vinula L., Pemphigus borealis Tulgren, Pemphigus spirothecae Pass., Melanophila picta Pall., Monosteria unicostata Muls and Rey, Phloemyzus passerini Sign., Melasoma populi L., Capnodis miliaris klug., Nycteola asiatica L., Lithocolletis populifoliella Tr., Salicicola kermanensis Lndgr., Empoasca decedens Paoli, and Chaitophorus leucomales Koch. All of these pests have been distributed in the entire poplar plantation in West-Azerbaijan province, but the highest species richness was recorded in Uremia (with Mediterranean climate) where 26 Populus clones were recorded. The most important point is that the role of poplar clones and climate of regions should not be ignored in distribution of

Keywords:*Pests, Population, Richness, Populus*

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Ecology

P3

Distribution of green oak moth, *Tortrix viridana* L. (Lepidoptera: Tortricidae)

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To determine the rate of the infestation of T. viridana in Ghabre-Hossein, Pardanan, Mirabad, Rabat, Banokhalaf, Ghoulak, Dare-ghabr and Khezerabad regions in 2011, random sampling was performed out of 40 trees of various Quercus genus per region. Four branches (50-cm long) from four cardinal directions were cut off as units of sampling for survey on green oak moth larvae and after counting, the larvae were transferred to the laboratory. Larvae of the last instars were separated based on their host oak species and were reared till their entrance to pupa stage. The weight of four-day-old female pupae was measured. Also, the distribution of the pest was determined through installation of five pheromone traps in different regions. Results showed that the trapped adult moths indicated the existence and distribution of this pest in the regions like Ghabre-Hossein, Pardanan, Mirabad and Ghoulak. But, T. viridana was not seen in Rabat, Dare-ghabr, and Banokhalaf regions (more than 1300 meter higher than free sea level with the cold climate). The highest and the lowest rates of infestation in the regions with severe infestation were recorded on Q. infectoria and Q. libani, respectively (df= 2; P<0.01). The collected pupae on Q. infectoria were the heaviest (df= 2; P<0.05). Also, among the infested areas, Pardanan and Khezerabad with 81% and 54%, respectively, were identified as the most and the least infested regions.

Keywords:*Population, Tortrix moth, Infestation, Quercus spp*

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Ecology

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Introduction: Separation of light from darkness

Gunther Pass
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About 15 years ago molecular data began to shake up classical ideas on the phylogenetic relationships of major arthropod subtaxa. One of the greatest controversies it has provoked concerns the origin of insects. While for a long time a close relationship between insects and myriapods was the common tenet, the new data points to a close relationship of insects with crustaceans, or to put it more succinctly, the insects are probably a terrestrial subset of crustaceans. Out of these fertile controversies a series of exciting phylogenetic research has sprouted. Several studies have shown that not only the traditional hypotheses on the origin of Hexapoda, but also their monophyletic status must be questioned. Further dispute refers to the earliest branches of the hexapod tree and a plethora of competing hypotheses have been published so far. As a consequence the ancestral phylogeny of hexapods became a case topic to study the problem of deep nodes which are extremely difficult to resolve. After years of intensive attempts to clarify the conflicting hypotheses it is now time for a "separation of light from the darkness" by critical discussion of all available data. The organizers of the symposium have attempted to bring together the leading experts from all fields dealing with the origin and early splits of hexapods. We can expect a comprehensive update of all relevant results to this topic and look forward to many enlightening discussions.

Keywords:*hexapods, apterygotes, phylogeny*

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Ecology

P3

Phylogenomic data support Remipedia as closest relative to Hexapoda

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Remipedes are a small and enigmatic group of crustaceans, first described only 30 years ago. The stygiobiontic species of this group dwell in remote underwater cave habitats, so called anchialine caves. New analyses of both morphological and molecular data have recently suggested a close relationship between Remipedia and Hexapoda instead of the former hypotheses that remipedes represent a rather basal split within crustaceans. Thus, remipedes may be pivotal for understanding the evolutionary history of both crustaceans and hexapods. However, to test this hypothesis using new data and new types of analytical approaches from the field of NGS (Next Generation Sequencing) data is important. The most recent phylogenomic analysis of pancrustaceans includes all crustacean species for which EST data are available (46 species), and the largest alignment encompasses 866,479 amino acid positions and 1,886 genes. A series of phylogenomic analyses was performed to evaluate pancrustacean relationships. This included a significantly improved quality of the data for predicting putative orthologous genes and for generating data subsets by matrix reduction procedures, thereby improving the signal to noise ratio in the data. The results demonstrate that the different ways to compile an initial data set of core orthologs and the selection of data subsets by matrix reduction may have marked effects on the reconstructed phylogenetic trees. The inferred phylogenies strongly support Pancrustacea with Remipedia as the sister group to Hexapoda.

Keywords:*phylogeny, origin hexapods*

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Ecology

P3

Origin and early splits in hexapods: A multidisciplinary approach within the international initiative 1000 Insect Transcriptome Evolution (1KITE)

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Starting about 400 million years ago, the evolution of hexapods is the most impressive success story in the animal kingdom in terms of species richness, diversification and adaptive radiation. While morphological diversity is generally high among the main groups of insects, disparity is highest when comparing entognathous groups with ectognathous lineages. Disparity is likewise high among crustacean subgroups discussed as the potential sister group of Hexapoda. These factors impede the reconstruction of the early splits and consequently of the early evolution of hexapods. Five primarily wingless groups are traditionally subsumed in an assemblage denominated "apterygotes" or 'basal hexapods': Collembola, Protura, Diplura, Archaeognatha, and Zygentoma. In the era of phylogenomics, basal hexapods are prone to be highly underrepresented. Embedded into the recently started 1KITE project which aims to sequence and analyze 1000 insect transcriptoms, working groups of 3 universities (Austria, Germany, and Japan) focus on the relationships and evolution of primarily wingless insects. With about 100 taxa final aim of this project is to analyze the largest taxon sampling concerning the basal hexapod lineages. Phylogenetic questions to be tested encompass: (i) monophyly of Hexapoda and Entognatha, (ii) sister group relationships among the entognathous groups (Collembola, Protura, Diplura) (iii) the sister group of the Hexapoda, and (iv) the phylogenetic position of the living fossil Tricholepidion gertschi. We will analyze not only molecular data sets but also investigate the reliability of these results with new data from developmental, embryological and morphological studies. First results will be presented along with focus, aims and workflow.

Keywords:*Hexapoda, EST, Phylogenomics, Entognatha*

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PS4TH001

IPM

P4

Contact toxicity of three formulations from lambda- cyhalothrin against German cockroach, *Blattella germanica* (L.) (Blattaria: Blattellidae)

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The German cockroach, Blattella germanica (L.) is a major household and public health pest worldwide. Current control strategies rely heavily upon various formulations of insecticides. In the present study, contact toxicity of three lambda-cyhalothrin formulations, emulsifiable concentrate (ICON 5 EC®), capsule suspension (DEMAND 10 CS®) and wettable powder (ICON 10 WP®) were tested against 5 to 7-day-old adults of the German cockroach exposed for 1 h. Experiments were conducted at 27±2 °C and 60±10% R.H with a photoperiod of 12:12 h (L:D). The results indicated that mortality of adults increased with increasing insecticide concentration. Males were more susceptible to all formulations than females. The most potent formulation was WP with the LC50 values of 9.87 and 12.24 mg.ai/㎡ against males and females, respectively. The formulation of CS attained the LC50 values of 11.02 and 13.42 mg.ai/㎡ and that of EC attained the values of 12.30 and 15.01 mg.ai/㎡ against males and females, respectively. The results demonstrated that the formulations of WP and CS had high activity against the German cockroach.

Keywords:*Blattella germanica, Contact toxicity, Formulations, Lambda-cyhalothrin*

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PS4TH002

IPM

P4

Natural enemies of *Bemisia tabaci* on cabbage plants in Qassim, Saudi Arabia

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Cabbage (Brassica oleracea Linn.) is considered one of the most important vegetable crops in Saudi Arabia. Cabbage agro-ecosystem formed a special ecological niche for a lot of insects, of which the sweetpotato whitefly (Bemisia tabaci, Genn.) is considered a destructive and a key pest of cabbage at Qassim region, KSA. There are three groups of natural enemies found associated with B. tabaci in cabbage. Predatory insects such as Chrysoperla carnea Stephens, Macrolophus caliginosus Wagner (Miridae: Hemiptera), Orius spp. (Anthocoridae: Hemipetra) and Coccinella undecimpunctata L. (Coccinellidae: Coleoptera) which attack B. tabaci immatures in cabbage fields. Chrysoperla carnea was the most abundant and efficient predatory insect species under field conditions, followed by M. caliginosus and Orius spp. compared with C. undecimpunctata. Only, Eretmocerus mundus Mercet (Aphelinidae: Hymenoptera) a parasitoid of B. tabaci was also observed at cabbage fields. This parasitoid had a higher population density and great impact in reducing B. tabaci population under field conditions. Moreover, an entomopathogenic fungi Cladosporium, was also observed infecting nymphs and adults in the field. Natural incidence of Cladosporium spp. was very high and caused a significant natural mortality among B. tabaci life stages. Despite the diversity of insect natural enemies and their population abundance, their effectiveness is still very low in the field. This may be due to the mutual inference effects among natural enemies, or may be the efficiency of insect biotic potential.

Keywords:*Cabbage, Sweet potato whitefly, Bemisia tabaci, Aleyrodidae, Biological control, Predators, Parasitoids, Entomopathogenic fungi*

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PS4TH003

IPM

P4

Psyllids damages to cultivated plants and forest timbers in Cameroon.

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Jumping plant-lice or psylloids form a moderate-size group of Sternorrhyncha (Hemiptera). They feed on plant sap, usually from the phloem, and are generally highly host specific.The present work describes the interactions between twenty psyllid species and their hosts in Cameroon. The damage that each psyllid species caused on its host has been described. Phytolyma fusca induced closed galls on leaves and buds of Milicia excelsa. These galls persisted during larval stages and exploded during the emergence of adults. Leaves and buds thus attacked dried and degenerated. Pseudophacopteron pusilum (on Dacryodes edulis), P. fuscovenosum (on Deimbolia sp.), P. nothospondiadis (on Nothospondias letestui), and Trioza sp. (on Schefflera barteri) induced closed and isolated galls on leaves of their respective hosts. Trioza erytreae (on citrus), Trioza sp. (on Stephania abyssinica) and Trioza sp. (on Drypetes leonensis) caused pit galls or crypts on leaves of their respective host.. Colophorina sp. (on Deutarium macrocarpum), Afrotrioza sp. (on Bersama sp.), Symtomosa sp. (on Homalium letestui), Pseudophacopteron cuniculus (on Bligia unijugata), and Diclidophlebia xuani (on Ricinodendron heudelotii) induced galls resulting from the enrolment of young leaves that hold larvae and adults. Psyllinae sp. (on Pittosporum viridiflorum); Triozamia sp. (on Antiaris Africana); Paurocephala sp. (on Dombeya ledermannii); Heteropsylla cubana (on Leucena glauca); and Trioza messii (on Caloncoba welwitchii) produced large quantities of honeydew responsible for the burns of the leaf tissue. The egg laying insertion mode of Mesohomotoma tessmanni caused some wounds in the leaf tissue of cocoa. Pseudoeriopsylla sp. attacked roots of Ficus thonningii.

Keywords:*psyllids, damage, biodiversity, Cameroon*

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PS4TH004

IPM

P4

The application of powdery preparation "Erdust" against bread ground-beetle (*Zabrus tenebrioides*) on wheat crops

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The bread ground-beetle (Zabrus tenebrioides) doesn't have a wide spreading in Uzbekistan and it is met in hearths. The harmfulness of larvae of bread ground-beetle depends not only on their number but on the nature of settling of sowings. At the centralized setting, even at high number of larvae, the sowing is lost not on the whole area. At a solid settling the existence of 3-4 larvae 1 m 2 they create a create threat and needs special destructive measures. Proceeding from mentioned above the strategy of struggle against bread ground-beetle is planned in the following way: - the processing of centres of damages; - conducting the solid processing; - presowing processing of seeds. Before conducting a solid processing of the centres of damages there were determined the forders of spreading larvae of bread ground-beetles, then there was conducted the processing with powdery preparation "Erdust" itsell counting 50kg/ha. The biological effectiveness of the preparation computed according to generally accepted methodics (in 3,5,7,10 days) was equaled to 93 %. On the plot with a solid setting of the pest there were used the preparation "Erdust" in the norm:50, 60 a 70 kg/ha. The best result was obtained in the variant with processing 70 kg/ha -92.9 %, which is higher than in variants 50 kg/ha (82 %) and 60 kg/ha (83,5) . The presowing processing was conducted by mixing moistented seeds of wheat with powdery preparation "Erdust" in the norm 15kg/t. The effectiveness of this method totaled 62,3 %.

Keywords:*Zabrus tenebrioides, bread ground-beetle, "Erdust", wheat, effectiveness*

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Genetic modeling of the fipronil resistance's increase in the population of Colorado potato beetle

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We examine genetic model of population resistance to insecticides in the case Leptinotarsa decemlineata Say and fipronil. We use data about resistance in the population of Colorado potato beetle on the South Ural and data from the experiment of testing minimal dose of fipronil. The model show a difference two season in increase resistance between variants with recommended and minimal doses. We added in the model second sign (colour morph of vertex and pronotum). It influence mediately on other-wintering of resistant individuals and it may be sign of latent death this individuals. Model with two signs show more difference in increase resistance between variants with different doses. This model would to predict time of increase population resistance more exactly. We examine in the model variants with minimal dose of two insecticides alternating with each other. Model show the slowest increase of resistance. And we suppose this variant is optimal way of pest management in the case of Colorado potato beetle.. Investigations funded by RFBR, grants No 11-04-01886-r_povolzhie_a and 11-04-97022-a.

Keywords:*resistance menedgment, genetic modeling, Leptinotarsa decemlineata, fipronil*

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P4

Examining the spatial and temporal factors that facilitate the distribution of *Halyomorpha halys* (Insecta: Hemiptera) in New Jersey, USA

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The brown marmorated stink bug (BMSB), Halymorpha halys (Stal), is an invasive pest of multiple fruit and vegetable crops in the mid-Atlantic region that has caused significant reductions in crop yield. It is native to China, Korea and Japan and was first found in the United States in Allentown, Pennsylvania in 1996. Since then, populations have been found in multiple states in the mid-Atlantic region, Midwest, and west coast, including but not limited to California, Michigan, New Jersey and Virginia. In New Jersey, the stink bug was first caught in 1999 in a black light trap. Additional catches have been collected and documented every year at geo-referenced traps across the state. This data will be displayed using Geographical Information Systems (GIS) to develop maps of distribution and density, with specific attention to population hot spots. These maps will provide an update on the movement and spread of brown marmorated stink bug and how these may be linked to nearby landscape and weather conditions.

Keywords:*Brown marmorated stink bug, Halymorpha halys*

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Influence of weed management on occurrence of the two-spotted spider mite, Tetranychus urticae and the native predacious mite, Neoseiulus womersleyi in apple trees in northern Japan

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To establish methods for successful IPM of Tetranychus urticae in orchards, the effect of weed management on the occurrence of this spider mite and Neoseiulus womersleyi on apple trees was investigated in 2009 and 2010 in an apple orchard in Akita Prefecture, northern Japan. Three plots differing in weed management were established in mid-April 2009; plot A: white clover sown and no mowing; B: mowing; C: no mowing. The chemicals sprayed were fungicides and insecticides that should not have affected the occurrence of both species. Mean number of T. urticae adult females on apple trees in plot C was significantly less than that in plot B in both years, and the number in plot A was significantly less than that in plot B in 2010. Neoseiulus womersleyi suppressed the increase of T. urticae on apple leaves in these plots when the density of T. urticae was low. The ground temperature during the daytime was comparatively stable in plot A and C, however that in plot B changed considerably. The maximum temperature on the ground in July in plot B exceeded 50°C. These observations suggest the possibility that the environmental conditions would remain suitable for T. urticae in vegetation not mowed and their immigrations into apple trees would be more reduced.

Keywords:*Tetranychus urticae, Neoseiulus womersleyi, weed management, apple*

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P4

Efficacy of bio-pesticides for the management of of *Helicoverpa armigera* on chickpea in Central India

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Pod borer, Helicoverpa armigera is a devastating pest of chickpea. We evaluated different combinations of biopesticides and natural plant products for the management of this pest in central India between 20008 - 2010. The treatments included: cow dung ash (CDA) (@40 kg/ha), garlic + tobacco leaf powder (GT) (500 g + 300 g), neem fruit powder extract (NFPE 5%), lime + cow dung + tobacco (LCT) (500 g + 15 kg + 5 kg) and H. armigera nuclear polyhedrosis virus (HaNPV). Untreated plots served as a control. All the treatments resulted in a significant reduction in H. armigera larvae compared to the untreated control. After three applications of bio-preparations/natural plant products, plots treated with HaNPV showed significantly greater reduction in larval population as compared to the plots treated with LCT. Plots treated with HaNPV, LCT, and NFPE 5% resulted in a significant reduction in pod borer damage as compared to the plots treated with CDA, and GT. The minimum pod damage 23.90% was recorded in plots treated with HaNPV, followed by the plots treated with LCT (27.03%), while NFPE 5% treated plots suffered 32.15% pod damage. The untreated plots suffered 54.59% pod damage. Highest yield of 17.46 q/ha was recorded in case of HaNPV treated plots, followed by 16.29 q/ha in plots treated with LCT, and these were significantly superior to the untreated control (11.43 q/ha). Based on these studies, HaNPV and LCT can be used for the management of H. armigera under moderate levels of pest attack.

Keywords:*Chickpea Helicoverpa armigera, IPM*

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IPM

P4

Effect of selected doses of neem and pyrethrum based bio-pesticides on parasitism of *Liriomyza huidobrensis* (Blanchard) by *Phaedrotoma scabriventris* Nixon

Everlyne S. Namikoye[1], Adenirin Chabi-Olaye[2], Komi K. M. Fiaboe[3], Daisy Salifu[4], Callistus K. P. O. Ogol[5]

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Liriomyza huidobrensis (Blanchard) is an important pest in vegetable production system in Kenya. Small scale farmers' routine application of synthetic pesticides induces resistance in the pest but decimate the natural enemies' complex. This study was carried out to evaluate the effect of neem (achook & neemroc) and pyrethrum based bio-pesticides on L. huidobrensis and its exotic parasitoid Phaedrotoma scabriventris Nixon. The three bio-pesticides significantly affect the adult L. huidobrensis' feeding and the survival rates of 1st and 2nd larval instars but had no effect on its oviposition and 3rd larval instar. The control registered the highest adult feeding and 1st and 2nd larval instars survival with 78.7±0.03 punctures/cm2, 90.0±1.7% and 92.0±6.6% respectively. The lowest feeding and survival rates of L. huidobrensis were recorded with Pyrethrum. Significant effects were recorded for doses ≥ 0.1, 0.5 and 0.5 for Achook, Neemroc and Pyrethrum respectively. The exposure of the 3rd larvae instar of L. huidobrensis to P. scabriventris with subsequent spray of respective bio-pesticides resulted in significantly higher pupal mortality compared to bio-pesticide use alone. While in the control, pupal survival and parasitism rates as high as 70.4±2.4% and 58.3±3.6 were obtained, at high concentration of Achook (0.25), they were as low as 11.1±1.1 and 8.0±1.5. The most suitable doses for a combined effect of the bio-pesticides and P. scabriventris were recorded at 0.05 for Achook, 0.25-0.5 for Neemroc and 0.125-0.5 for Pyrethrum. This suggests possibility of sustainable combination of bio-pesticides and the parasitoid P. scabriventris in the control of L. huidobrensis.

Keywords:*IPM, Bio-pesticides, Liriomyza huidobrensis, Phaedrotoma scabriventris, Feeding, Survival*

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Suppression of fecundity, vitellin content and Nlvg gene expression in *Nilaparvata lugens* Stal (Hemiptera : Delphacidae) adult females exposed to sub-lethal dosages of indoxacarb and chlorantraniliprol

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The brown planthopper, Nilaparvata lugens (Stal) (Hemiptera: Delphacidae), is a classic example of a pest that experiences insecticide-induced fecundity with such compounds as pyrethroids (deltamethrin) and organophosphates (triazophos). The insecticides indoxacarb and chlorantraniliprole were recently developed with high insecticidal activity in the control of lepidopteran insects, including Tryporyza incertulas (walker) and Cnaphalocrocis medinalis (Guenee). In this study, we examined the changes in the fecundity of N. lugens, the vitellin contents in the fat bodies and ovaries and the vitellogenin (Nlvg) gene expression of adult females exposed to these new insecticides. Our findings showed that sub-lethal dosages of indoxacarb and chlorantraniliprole significantly suppressed the fecundity of the females. Treatment with 10, 20, 40 and 80 ppm indoxacarb decreased Nlvg mRNA expression by 35.4%, 74.4%, 79.6% and 61.9%, respectively, and treatment with 7.5, 15, 30, 60 and 80 ppm chlorantraniliprole decreased Nlvg mRNA expression by 79.3%, 40.2%, 40.0%, 79.8% and 51.8%, respectively. The vitellin contents in both the fat bodies and ovaries of the adult females also decreased, indicating that the two insecticides suppressed the synthesis of vitellin in the fat bodies and the transfer of vitellin to the ovaries. This decrease in the vitellin contents in the fat bodies and ovaries may be attributed to the low Nlvg expression. The study of the effects of indoxacarb and chlorantraniliprole on fecundity should have significance for the wide usage of these new insecticides in rice plants. Indoxacarb and chlorantraniliprole are ideal alternative insecticides to control T. incertulas and C. medinalis in China.

Keywords:*indoxacarb, chlorantraniliprole, Nilaparvata lugens, fecundity, vitellogenin mRNA gene expression*

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P4

Effects of commonly used synthetic pesticides in Pea production systems on *Liriomyza huidobrensis* and its parasitoids in Central Kenya

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Dimethoate, Dynamec, Thunder, Cyclone, Bestox, Folicur, Milraz and Bulldock are among the pesticides routinely used in the Pea production system in Central Kenya. Field and laboratory studies were carried out to assess their implication on the management of Liriomyza huidobrensis (Blanchard). On-farm field investigations consisting three management practices: farmers' routine use of pesticides (FP), reduced pesticide use (RP) and no pesticide use (CP) were conducted. Laboratory experiments were designed to test the effect of the pesticides on L. huidobrensis and two parasitoids Diglyphus isaea Walker and Phaedrotoma scabriventris Nixon. Liriomyza huidobrensis was more abundant in FP than CP and RP. Despite the constant presence of the pest, local parasitoids were only sporadically recorded, with CP recording higher numbers of parasitoids. Bestox, Cyclone and Dynamec were efficient in the laboratory against adult leafminer and their estimated LC50 (0.32, 0.5 and 0.24 ml/l respectively) were below the recommended doses. None of the pesticides were successful in controlling leafminer larva and the LC50 for Bestox, Bulldock, Dimethoate, Dynamec and Thunder (1.41, 1.64, 2.91, 1.16 and 2.94 ml/l respectively) were all above their recommended doses. Apart from Thunder which required 0.80 and 0.77 ml/l for LC50 for D. isaea and P. scabriventris respectively, all the pesticides were detrimental to the parasitoids at dosages below the recommended. This study demonstrated that routine pesticide use in farmer fields hampers effective control of L. huidobrensis since the pesticides eliminate the natural enemies without having any effective control on the larval stage of the pest.

Keywords:*Horticulture, Leafminer flies, Liriomyza huidobrensis, Pesticides, Parasitoids, Abundance*

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Utilization Package of biological control agent of the green lacewing, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae)

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Study on the packages of the green lacewing Mallada basalis (Walker) (Neuroptera: Chrysopidae), aimed to find out the more applicable package for the release of this predator species for biocontrol. The primary trial was designed in 2×3×5 factorial experiment in completely randomized design with 25 replications, under laboratory condition at 25+2 OC, 75+2 % RH. The three factors were, factor I: tubes (0.5 cm in diameter x 7 cm in length) made of two types of material for confining the predatory eggs (1) plastic tube (2) white opaque A4 paper tube; factor II: two sizes of vermiculite and a mixture of the vermiculite, filled in the tubes (1) coarse (2) fine and (3) a mixture of coarse and fine (1:1) and factor III: five densities of eggs of the green lacewing, (1) 50 (2) 100 (3) 150 (4) 200 and (5) 300 eggs, enclosed in one either plastic or paper tube/replication. Results obtained showed that the main effects -type of material and egg density were not statistically significant (P>0.05), but size of vermiculite was (P<0.05). Only interaction of the main effects occurred between the main effects type of material and size of vermiculite. Hence, further analysis of variance indicated that mixture of coarse and fine vermiculite (1:1) in either plastic tube or paper tube gave the better hatching of eggs of M. basalis with 40.46±8.79 and 44.02±7.62%, respectively

Keywords:*Packages, Mallada basalis (Walker), biological control, vermiculite*

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P4

Fumigant activity of plant essential oils from basil and spearmint on the Mediterranean flour moth *Anagasta kuehniella* Zeller (Lepidoptera : Pyralidae)

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The fumigant activity of essential oil vapours distilled from sweet basil Ocimum basilicum and spearmint Mentha spicata (Lamiaceae) were tested against the Mediterranean Flour Moth. Various oil doses (5, 50, 250 and 500μl/lt air) and exposure times (0.5, 1 and 2 days) were tested during the present study. The essential oils were subjected to GC-MS analysis and revealed that the major compounds were for spearmint oil carvone (66.1%) and limonene (25.2%) and for basil oil estragol (71.7%) and linalool (17.7%). Generally, no significant differences on insecticidal action were noted between basil and spearmint oil. Both oils were highly effective against adult moths, given that 100% mortality was recorded after exposure to the lowest dose (5 μl/lt) for the shortest time interval (0.5 day). Noteworthy egg mortality was also recorded reaching at 73% for basil and 66% for spearmint. Toxicity data indicated that larvae and pupae were the most tolerant stages in all cases. Larval mortality never exceeded 30% and 26%, for basil and spearmint, respectively. Basil and spearmint oils managed mortalities as high as 31% and 21% in pupae, respectively. LC50 and LC99 (for 1 day exposure) as well as LT50 and LT99 (for each oil dose) values were estimated via probit analysis. Developmental stage proved to be a significant factor whereas the effect of oil species on insect mortality was insignificant. Basil and spearmint oils did not show satisfactory overall insecticidal activity against A. kuehniella, demonstrating much less toxicity than other essential oils from aromatic plants.

Keywords:*essential oils, Anagasta kuehniella, Ocimum basilicum, Mentha spicata, fumigant activity*

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P4

Diversity and seasonal activity of bark and ambrosia beetles in South Carolina, USA

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Clemson University, U.S.A.

Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are important pests of horticultural, agricultural and forestry crops around the world. The diversity of the bark and ambrosia beetles in South Carolina, USA, is known but their seasonal activities are not. This study compared the diversity and seasonal activities of bark and ambrosia beetles in 3 distinct habitats in South Carolina - ornamental tree nursery (low plant diversity, low stand density), botanical garden (high plant diversity, low stand density) and pine-hardwood forest (high plant diversity, high stand density). Although the abundance of bark and ambrosia beetles captured in traps baited with ethanol and alpha-pinene was higher in the pine-hardwood forest, the diversity and seasonal activity were similar across the habitat types. Some species were active throughout the year but most species had distinctive peaks in their activity, typically in the spring or summer. Results from this study may help to improve the prediction of the flight activity of important bark and ambrosia beetle pests and the prevention of their attacks on economically important crop species.

Keywords:*Bark beetle, ambrosia beetle, ornamental trees, forest*

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P4

The economy of a suscessful ambrosia beetle management program in ornamental tree nursery

Juang Horng Chong
Clemson University, U.S.A.

The granulate ambrosia beetle, Xylosandrus crassisuculus (Motschulsky), is one of the most damaging insect pest species of ornamental tree nurseries in the southeastern United States. A management program based on moitoring with semiochemical and preventive applications of pyrethroid against this pest species was implemented in an ornamental tree nursery in Georgetown, South Carolina, USA. The management program reduced the percentage of susceptible trees damaged by the granulate ambrosia beetle from 13% in 2009 to 0.13% in 2010. The frequency of applications was reduced by 25% in 2010 due to a more precise timing of the applications. When the costs of control (material, labor and others) and damage were taken into account, the cost-benefit ratio of this program was 1:20. A similar program has been adopted for additional nurseries in the area.

Keywords:*Ambrosia beetle, semiochemical, pyrethroid, cost-benefit analysis*

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Geographic variation among Colorado potato beetle populations and its implications for managing insecticide resistance

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Colorado potato beetle is the most important insect defoliator of potato in North America. It is an evolutionary plastic species, capable of adaptation to a variety of adverse conditions, including insecticide applications by humans. Management recommendations are often developed based on studies of as little as one Colorado potato beetle strain, often maintained under laboratory conditions over extended periods of time. A better understanding of geographic variability among Colorado potato beetle populations may eventually allow customizing control techniques to local conditions. Population growth potential, propensity for cannibalism, and levels, inheritance, and mechanisms of resistance to a commonly used insecticide imidacloprid were quantified under shared laboratory conditions in strains from different geographic locations. The differences in the number and size of produced egg masses, egg hatchability, and pre-oviposition period were detected among the strains. One of the strains had a female-skewed sex ratio, which is the first such record for this species. Not surprisingly, strains with high fecundity and low hatch rate had high cannibalism. There was a considerable variation in the strength of imidacloprid resistance among the locations. However, resistance had a similar monooxygenase-dependent mechanism (P450s) in all tested strains, and its inheritance was closer to recessive than to additive. Measured life history parameters did not correlate with strain resistance to insecticides. Geographical isolation appears to explain the observed variation.

Keywords:*integrated resistance management, population growth, cannibalism, P450*

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Acaricidal bioactivity against *Tetranychus cinnabarinus* (Acari : Tetranychidae) of *Artemisia annua* extracts and their phytotoxicity on pest mites host plant cowpea

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The acaricidal bioactivities of Artemisia annua extracts against the carmine spider mite Tetranychus cinnabarinus were confirmed. These extracts were the parallel and sequenced extracts of different solvents from the root, stem and leaf of Artemisia annua. And we found that the leaf acetone parallel extract showed the strongest acaricidal bioactivity, treatment after 48h at the concentration of 5mg/mL killed all test mites and the median lethal concentration (LC50) at 48h was 0.4341mg/mL. However, in order to clarify the toxic effects of these plant extracts on the seed germination rate and the radicle and hypocotyl growth inhibition of cowpea, which was principal host plant of the carmine spider mite. The concerned phytotoxicity tests against cowpea were also carried out in laboratory. The results showed that the impact of cowpea seed germination of Artemisia annua roots, stems and leaves extracts varied with the solvent species and different extraction methods. Of which the impact of stem ethanol sequenced extract against cowpea seed germination rate was greatest, and showed significant inhibition effect, and the germination rate was only 23.81%. Otherwise, the leaf acetone sequenced extract also showed strong inhibition effect on cowpea seed germination rate, of which the germination rate was 40.48%. The different solvent extracts of root stem and leaf of Artemisia annua in July were tested their phytotoxicity against cowpea seeds, and the results showed that weak inhibition effect on radicle and hypocotyl growth, and maybe also exist some material which promoting radicle, hypocotyl growth.

Keywords:*Artemisia annua,Tertanychus cinnabarinus,phytotxocicity,seed germination*

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PS4TH018

IPM

P4

Cost-effectiveness of integrated pest management compared with insecticidal spraying against the German cockroach in apartment buildings

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This study assessed the cost and effectiveness of an integrated pest management (IPM) program using hydramethylnon gel bait compared with conventional spraying method for controlling the German cockroach, Blattella germanica (L.) (Blattodea: Blattellidae), in two residential buildings in Yasuj, Iran. The IPM approach was based on educational programs using pamphlets, posters and lectures, sanitation using vacuuming and application of hydramethylnon gel bait. Conventional approach used spray of cypermethrin (10% EC) on baseboard and cracks-and-crevices. Sticky traps were used as tools for monitoring cockroach populations throughout 15 weeks treatment periods.The IPM approach reduced (943%) the rate of insecticide application compared to the conventional spray. Cockroach populations in the IPM treatment were significantly reduced from an average of 12.2 ± 3.01 cockroaches per unit before treatment to zero cockroach per unit by week four and thereafter. Cockroach populations in the conventional spray treatment were reduced from an average of 11.5 ± 4.43 cockroaches per unit before treatment to an average of 3.4 ± 0.99 cockroach per unit after 11 weeks of post treatment. The IPM treatment improved 100% of infested units compared to 78% for spray treatment to obtain a clean level of infestation (<1 cockroach per trap per unit). The results suggest that the intervention by IPM using hydramethylnon gel baits significantly reduced cockroach infestation compared to cypermethrin spray throughout the 11 weeks of post-treatment period. However, within the study period, the IPM system involving gel baits, educational program and sanitation was 363.2% more expensive than the conventional method.

Keywords:*Blattella germanica, IPM, Hydramethylnon, cost comparison*

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PS4TH019

IPM

P4

Contact activities of extracts from different parts of *Phytopacca americana* L. against *Tetranychus cinnabarinus* (Boisduval) (Acarina:Tetranychidae)

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The different solvent extracts from different parts of Phytolacca americana L. were tested for their acaricidal activities against female adults of carmine spider mite, Tetranychus cinnabarinus (Boisduval), by slide-dip bioassay. The results indicated that acetone extract of the root was more active than that from leaves and stems at 48 h and 72 h after treatment, and other solvent extracts from any organs, and it exhibited strongest activity against female adult, causing 95.72% to 100.00% mortality in 72 h at the concentration of 2.5 mg?ml-1 after treatment, meanwhile, the LC50 values of eggs and female mites were 2.1637 and 7.1032 mg ml-1, respectively. The acetone root extract was fractionated using a silica gel column, after thin layer chromatography, the concentrated extract was separated into 15 groups of fractions and further tests for their acaricidal activities were conducted. Fraction 9 was found to possess higher acaricidal activity. At the concentration of 2 mg ml-1, the corrected mortalities of the fraction 9 against adult mites were 60.84% and 95.75% in 12h and 24h after treatment, respectively. The LC50 values of the fraction 9 were 1.2609, 0.2035 and 0.1026 mg ml-1 after treatment 24h, 48h and 72 h, respectively. The 9th group was identified as one potent acaricidal saponins through the phytochemical test. From the qualitative determination of the saponins, it is concluded that the acaricidal saponins is a type of oleanane triterpenoidal saponins.

Keywords:*Phytolacca americana L., Tetranychus cinnabaribus, Acaricidal activity, plant extract, saponins*

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PS4TH020

IPM

P4

Bio-ecology of the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae)

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The red palm weevil, Rhynchophorus ferrugineus, is the most destructive pests of palms in the world. In the Mediterranean the Canary date palm, Phoenix canariensis, has become the main host of this weevil. For this reason we carried out a study aimed at determining the life cycle of R. ferrugineus in live P. canariensis palms. Egg lethal temperature threshold and thermal constant were determined and resulted in 13.10ºC and 40.4 DD, respectively. A semi field assay was carried out in a mesh enclosure where living P. canariensis palms were artificially infested with neonate larvae under natural conditions. Maximum mortality rates for R. ferrugineus were observed for palms infested in December and January, whereas those infested from April through September showed maximum survival rates. Mean monthly temperatures below 10.30ºC were lethal for neonate larvae, as 4.50ºC were for older instars. All recovered larvae could be classed according to one of thirteen instars. A thermal constant of 666.5 DD was estimated for complete larval development. Pupal development required an additional 282.5 DD. To better understand the phenology of R. ferrugineus populations in the Mediterranean basin, we have estimated the effect of temperatures within the range 10-25ºC on the reproductive parameters of R. ferrugineus. Our results confirm that oviposition is strongly affected by temperature. Thresholds for oviposition and egg hatching (15.45º and 13.95ºC, respectively) are very close to mean annual temperatures registered in most of the northern shore of the Mediterranean basin. These results should help refining existing control strategies against this pest.

Keywords:*Phoenix canariensis, life cycle, temperature thresholds, Thermal constant*

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IPM

P4

Ground cover management affects species composition, distribution, and seasonal abundance of Thysanoptera occurring in citrus.

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There is a complex of Thysanoptera species inhabiting citrus and some of them are considered pests. This study was aimed at studying the effect of ground cover management on thrips species composition, distribution and seasonal abundance in commercial clementine mandarin orchards. Three soil management systems were compared: resident vegetation cover, Festuca arundinacea Schreber (Poales: Poaceae) cover, and bare soil. The ground cover influenced the abundance of some thrips in the cover. Among the main species causing fruit damage in citrus, we found Frankliniella occidentalis (Pergande) and Thrips tabaci Lindeman. The F. arundinacea cover strongly depressed the abundance of these thrips species in ground cover vegetation. The resident vegetation cover could provide a range of suitable hosts supporting populations of potential thrips pest species out of the main citrus peak blooming period. Apart from that, the F. arundinacea cover, which hosted Poaceae-specific thrips species, could provide alternative food enhancing populations of predatory phytoseiid mites which could reduce populations of potential citrus pests below economic injury level. Our results confirm the suitability of F. arundinacea as a cover crop for clementine mandarins

Keywords:*Conservation Biological Control, Frankliniella occidentalis, Thrips tabaci, Pezothrips kellyanus, Phytoseiidae, clementine mandarine*

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PS4TH022

IPM

P4

Life history parameters for *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) preying upon *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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The polyphagous predators Nesidiocoris tenuis Reuter and Macrolophus pygmaeus (Rambur) (Hemiptera: Miridae) are endemic natural enemies that commonly inhabit tomato crops in the Mediterranean basin. Both predators are mass-reared and, until recently, they were mainly released to control whiteflies. However, both mirids have been observed to prey efficiently on the eggs of the invasive tomato pest Tuta absoluta Meyrick (Lepidoptera: Gelechiidae). In this study, the life-history traits of these two predators were studied and compared when they were fed exclusively with eggs of T. absoluta on tomato plants. The experiments were performed under laboratory conditions (25 ºC, 60% RH and 16:8 h L:D). The immature survival for both mirids was similar (?90%), although the nymphal development time was significantly faster for N. tenuis than for M. pygmaeus (13.0 ± 0.1 d and 16.0 ± 0.2 d, respectively). The progeny was also higher for N. tenuis than for M. pygmaeus (38.9 ± 6.9 and 3.0 ± 1.7 nymphs per female, respectively). These differences resulted in higher selected life history parameters for N. tenuis when compared to M. pygmeus (e.g. the intrinsic rate of increase was 0.135 for N. tenuis and 0.006 for M. pygmaeus). Our results, may explain the higher efficacy of N. tenuis controlling T. absoluta and the increasing prevalence of N. tenuis in those tomato crops infested by T. absoluta.

Keywords:*South American tomato pinworm, progeny, development time, intrinsic rate of increase, biological control, tomato*

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P4

Molecular evaluation of sterile *Ceratitis capitata* males performance in field cages: Correlation of mate achievement and fruit damage avoidance

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In area-wide Sterile Insect Technique (aw-SIT) programs there is a lack of real-time information about how the success of the sterile male releases is translated in pest population reduction. Recently described field cage tests have demonstrated the possibility to relate the molecular sterile sperm ID in the spermathecae of C. capitata females with the fruit damage. Field cage tests were carried out to study the activity of sterile Vienna-8 (V8) males under six release ratios (1:0:0, 1:1:0, 1:1:1, 1:1:5, 1:1:10 and 1:1:20; wild females: wild males: V8 males) and their efficacy in population reduction under different environmental conditions in the Mediterranean coast of Spain. The data collected were the recaptured females in traps, sperm ID in the spermathecae of trapped females, and the viable progeny produced. Data showed the significant effect of temperature (as environmental variable) and sterile males release ratio correlated on the V8 performance (sperm transference and progeny reduction). During the winter neither was identified male sperm in spermathecae nor was obtained progeny. Out of the winter, the proportion of V8 sperm ID in spermathecae was similar for the ratios 1:1:10 and 1:1:20 which was the highest compared to 1:1:0 over all the temperatures tested. Moreover, the progeny reduction showed to be strongly related with the proportion of V8 sperm ID in females independently of release ratios and environmental conditions. The models obtained based on molecular sperm ID could contribute to enhance the measurement of the efficacy of SIT programs.

Keywords:*medfly, autocide control, molecular sperm ID*

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P4

Novel insect toxins from two scorpions inhabiting Japan, *Liocheles australasiae* and *Isometrus maculatus*

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Scorpions are venomous animals and many thousands of people are stung worldwide and hundreds are died in the world. Two scorpion species, Liocheles australasiae (Hemiscorpiidae) and Isometrus maculatus (Buthidae), inhabit the southern islands of Japan. Even though we have not suffered serious damage from them, scorpions are utilizing venoms to catch insects. To date, we isolated two toxins from L. australasiae (LaIT1 and LaIT2) and three toxins from I. maculatus (Im-1, Im-2, and Im-3) by repeating HPLC fractionation and monitoring the insecticidal activity against cricket. Primary structures of these toxins were determined using mass spectrometry and Edman degradation as well as cDNA cloning. LaIT1 is composed of 36 amino acids with two disulfide bonds and shows no sequence homology to any other known toxins. LaIT2 is of 59 amino acids with three disulfide bonds and homologous to β-KTx affecting potassium channel. Im-1 is linear peptide with 56 amino acids without cysteine residues. Im-2 is composed of 67 amino acids and Im-3 is of 63 amino acids, and both of them contain 4 disulfide bonds. The patterns of disulfide linkage of LaIT2, Im-2 and Im-3 are not analyzed yet. LaIT2 and Im-1 have also anti-bacterial activity. Im-2 and Im-3 are homologous to the toxins affecting Na-channel. Im-2 is excitatory toxins and also toxic to mammals, but Im-3 is depressant toxins. We analyzed the three-dimensional structure using NMR to find that the structure has the knotting peptide motif, and studied the structure activity relationship of LaIT1 and its analogs.

Keywords:*scorpion, toxins, Liocheles australasiae, Isometrus maculatus*

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P4

Botanical extracts in northeastern Brazil: Biological alternative to pest control in fruit species

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The agriculture is one of the activities with more impact over the environment. In northeastern Brazil, it has been evidenced the necessity to optimize the exploration system of some fruit species, with notability to the following cultures: cashew (Anacardium occidentale), mango (Mangifera indica), guava (Psidium guajava), papaya (Carica papaya) and coconut (Cocos nucifera). Research results show the botanical extracts efficiency, especially oils as alternative to chemical insecticides. The management of the coconut pest species Aceria guerreronis (Acari: Eriophyidae), Atheloca subrufella (Lepidoptera: Phycitidae) and Parisoschoenus obesulus (Coleoptera: Curculionidae) have been adopted successfully by the major coconut producing areas in Brazil, using the aqueous solution: cotton oil (1.5%) and neutral detergent (1.0%). Similar results were obtained in cashew controlling the whitefly, Aleurodicus cocois (Hemiptera: Aleyrodidae). The use of the plant oil mixture, cotton (1.5%), castor (1.5%) and neem (1.0%) and/or mineral oil (1.0%) in different interval of 3 and 4 weeks, has brought positive results in the control of Trizoida sp. (Hemiptera: Psyllidae), Frankliniella schultzei (Thysanoptera: Thripidae) and Tetranychus urticae (Acari: Tetranychidae), respectively, in guava, mango and papaya. The cost to control these pests reduced up to 75% and was noticed a fast recovery of the natural enemies population in the studied areas.

Keywords:*Coconut, mites, whitefly, Aceria guerreronis, Trizoida, Thrips*

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P4

Employing pheromone traps for seasonal monitoring of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) in Bulgaria

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Two year seasonal monitoring of the flight of Phthorimaea operculella by means of pheromone traps was organized at four sites in three districts in Bulgaria: Sofia, Kyustendil and Plovdiv. Comparison between sticky traps and dry funnel traps has shown that sticky traps were much more effective in capturing of males of this pest than the dry ones. The results of our investigations showed that the pest could appear in the field as early as the end of March and also intensive flight could be observed up to the end of November. Single catches were registered even in January. However, because of overlapping of the generations, their number and periods of moth emergence was not possible to be distinguished by the catches in pheromone traps. Analyses of climatic data (mean air temperature and mean rainfall) indicated that none of the climatic variables investigated strongly affected P. operculella trap catches. Acknowledgments: The research was supported by Grant DO02-244/2008 of the Bulgarian National Scientific Fund.

Keywords:*Phthorimaea operculella, pheromone traps, seasonal monitoring*

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P4

Optimum predation temperature and pesticide susceptibility of the predatory bug, *Geocoris proteus* Distant (Heteroptera: Geocoridae)

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The big-eyed bug Geocoris proteus Distant has been observed on Allium crops in western Shizuoka Prefecture. The bug is an important predator of several economic pests of various crops in the field. We investigated the optimum temperature for prey consumption and also the bug's pesticide susceptibility. First, adult and 3rd-instar larvae of G. proteus were provided with Thrips tabaci at 15, 20, 25, or 30 °C for 24 h. Geocoris proteus consumed the maximum numbers of prey consumed at 25 and 30 °C. (Adult female G. proteus preyed on about 80 2nd-instar larvae of T. tabaci.) These results suggest that G. proteus has high predatory potential and that high temperatures are suitable for predation. Second, G. proteus adults or 3rd-instar larvae were dipped into 66kinds of pesticide solution with practical concentration in labo. Two organophosphorus insecticides, 1 carbamate, 2 neonicotinoids, 2 insect growth regulators, 8 synthetics, 1 natural product based insecticide, 5 microbial insecticides, 4 acaricides, and 14 fungicides were rated as harmless (< 30% mortality) to the adults and 3rd-instar larvae. These pesticides are therefore compatible with G. proteus conservation in the field, and their use should be recommended in integrated pest management programs to control Allium insect pests.

Keywords:*Geocoridae, Optimum predation temperature, pesticide susceptibility*

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PS4TH028

IPM

P4

Individual-based model for the spread of citrus greening disease by the vector insect *Diaphorina citri*

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Citrus greening disease (Huanglongbing, HLB) is an untreatable insect-borne disease that makes trees withered and dies. The IPM system which is focused on the vector insect is required for reducing the economic loss. To establish an IPM system, risk assessment of the relevant hindrances is necessary. Applying simulation model to the estimation is one of the reasonable risk evaluation methods. Thus, we developed a model which estimates the spread of HLB by the vector insect Diaphorina citri. Until quite recently, almost all models of pathogens and hosts were developed within the framework of mean-field models, which assumed uniform distribution and interactions of the respective individuals. In recent years, however, the increased calculation speed of computers has enabled the development of an Individual-Based Model (IBM), wherein each individual, of the pathogen and the vector, behave individually, acting according to a predefined set of rules. IBM is able to examine disease-spread dynamics in consideration of space, by calculating the cumulative results of the individual behaviors. Hence, we used the framework of IBM to establish the HLB spread model. Our simulated results suggested that both delaying the transition from latent period to infectious period, and removing infectious citrus trees, suppressed the HLB spread rate in an orchard. Additionally, the results of our a preliminary trail suggested that cooperative control of D. citri on the part of orchard owners in a given area, reducing the number of virulent individuals was effective in suppressing the HLB spread rate.

Keywords:*vector, risk assessment, Individual-Based Model*

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P4

The association of a Lepidopteran borer complex between pecan nuts (*Carya illinoensis*) and citrus (*Citrus sinensis*) in the vaalharts region

Andre Petrus van Rooyen[1], Vaughn Richmond Swart[2], Sean Moore[3]

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The possible migration of economically important Lepidoptera borer species from citrus (Citrus sinensis) to pecan nuts (Carya illinoensis), and vice versa, at Vaalharts, Northern Cape is investigated. Possible species of interest within the lepidopteran borer complex, which are established pests on citrus and pecan nuts, false codling moth Thaumatotibia leucotreta (Meyrick, 1913) (Lepidoptera: Tortricidae) and carob moth Ectomyelois ceratoniae (Zeller, 1839) (Lepidoptera: Pyralidae). The incidence of the two lepidopteran species in pecan orchards, in proximity to citrus orchards, is compared to pecan orchards remote to citrus orchards. Approximately, 100 pecan nuts and 50 citrus fruit (from the trees and ground) were collected from adjacent orchards, 10 and 50 m from the edge of pecan and citrus orchards. Five farms that met these conditions were selected as study sites. The samples were placed into emergence boxes to allow the immatures to develop to adulthood. The incidence of the two lepidopteran species relative to the distance of the pecan and citrus orchards from each other will determine the extent to which the migrations may occur. The higher proportion of E. ceratoniae (55%) was recorded in the pecan study sites proximal to citrus orchards, with a lower incidence (45%) in the remote pecan control sites. Citrus orchard study sites had incidence of only E. ceratoniae. Thaumatotibia leucotreta was present in two of the pecan orchards. Ectomyelois ceratoniae is the dominant of the two pest species under investigation. Proximity of citrus orchards don't seem to increase the incidence of E. ceratoniae in pecan orchards.

Keywords:*Carob moth, false codling moth, incidence, migrate, pecan nuts, citrus,*

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P4

Integration of bean resistance and a botanical insecticide (neem formulation) to manage the Mexican bean weevil.

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Considerable losses occur in stored beans in Brazil due to the attack of key pests such as Zabrotes subfasciatus (Coleoptera: Bruchidae), which is able to cause cross-infestation of grains. The goal of this work was to evaluate the preference of Z. subfasciatus by six different kinds of beans belonging to the genus Phaseolus, Vicia and Vigna and how a neem formulation could help preventing damage in the tested grains. In order to achieve that, the six tested genotypes were arranged in an arena inside of a cage and 60 non-sexed insects were released in the center. Coffee cups containing 20g samples and arranged in a completely randomized design with five replicates were evaluated for the number of insects 0.5, 1, 2, 4, 6, 12, 22, 30 and 48h after the initial release. The number of damaged grains was evaluated at the end of the essay. The data were analyzed by repeated measures analysis and the results were used to select the genotypes to be further tested in three other bioassays using the neem formulation at 10% of concentration and water as control. The results showed that while the genotype 'fradinho (Vigna unguiculata)' was the most preferred followed by 'vinagre (Phaseolus vulgaris), the genotypes 'preto (P. vulgaris)', 'fava amarela and fava rajada (Vicia faba)' and 'corda sempre verde (V. unguiculata) were the less preferred by Z. subfasciatus. The treatment with neem reduced the number of insects and damaged grains by almost zero even in the highly preferred genotypes.

Keywords:*Antixenosis, Azadirachta indica, stored pest.*

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IPM

P4

Adoption of multiples tactics of the cotton IPM to manage the boll weevil.

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The boll weevil (BW - Anthonomus grandis) is a highly harmful pest of cotton in Brazil where it is widespread and causes significant economical losses. This work aimed at evaluating the control of BW obtained by the association of plant resistance and mass trapping or chemical control with a botanical insecticide (neem formulation). For that purpose, natural colored cotton (BRS Rubi, Safira and Topazio) were cultivated isolated or surrounded by a white cotton (BRS Aroeira) which is known to be BW-resistant. Each plot was BW controlled by either mass trapping (one accountrap + pheromone/plot) or by spraying a neem formulation (at increasing concentrations of 0.5, 1 and 2%). The neem formulation was used every time that the infestation level (IL) reached 10%. Plots were weekly sampled, starting at 90 days after sowing. The association of the colored cotton Rubi with the white cotton Aroeira reduced the need to spray with neem in more than 50%. Although the same did not happen to Safira and Topazio, the IL was smaller in the plots cultivated with these varieties, surrounded by Aroeira and sprayed with neem. Concerning the mass trapping, the number of times BW reached the IL did not vary among the plots surrounded or not by the white cotton, but the IL was always smaller in the former and, mostly, smaller than in the same plots sprayed with neem. The number of squares and bolls damaged by BW was maximum in BRS Rubi and minimum in BRS Safira and Topazio.

Keywords:*Gossypium hirsutum, Azadirachta indica, rational management*

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IPM

P4

Attractiveness of blue and ultraviolet light-emitting diodes to *Thrips palmi* Karny

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To develop a method for physical control of Thrips palmi Karny, we investigated the ability of visible and ultraviolet light-emitting diodes (LEDs) to attract the insect. LEDs emitting blue (peak wavelength: 470 nm) and green (peak: 525 nm) visible light attracted significantly larger numbers of T. palmi than other LEDs. UV-LEDs (peak: 365 nm) were more efficient than blue LEDs. Mixing blue and UV LEDs did not increase the proportion of T. palmi trapped.

Keywords:*Thrips palmi Karny, LED, ultraviolet light*

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PS4TH033

IPM

P4

Energy-saving light trap for cigarette beetles using flickering UV-LED.

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Adult cigarette beetles exhibit positive phototactic response, especially to UV light, and can therefore be captured in light traps. The influence of the flicker-frequency of light on photoresponse has been demonstrated for some insect species, but no information exists for the cigarette beetle. This study examines the influences of light flicker-frequency and duty cycle on cigarette beetle capture using a light trap to evaluate the practical value of flickering UV-LED light for the cigarette beetle. In dark conditions, 100 adult beetles were released in a 1.8 × 3.6 × 1.8 (H) m chamber kept at 29°C. A water-pan trap (20 × 68 cm) equipped with a UV-LED lamp emitting light (375 nm) with a controlled flicker-frequency and duty cycle was used to catch light-lured beetles. No significant difference was found between catches achieved with traps emitting flickering light (50% duty cycle) at a flicker-frequency of 50, 100, 150, 200, 300, 500, or 1000 Hz and those with traps emitting non-flickering light. Furthermore, no difference in catches was observed between light traps that emitted non-flickering light and those emitting light with a controlled duty cycle (20, 30, 40, 50%; 200 Hz). Results indicate that light traps with flickering UV-LED (50-1000 Hz, 20% duty cycle) can be operated with 20% of the electricity consumption of non-flickering UV-LED traps without reduced attractiveness to cigarette beetles.

Keywords:*Lasioderma serricorne, light trap, UV-LED, flicker-frequency, duty cycle, energy-saving*

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P4

Effect of flonicamid on voltage-gated potassium currents

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Flonicamid is a pyridinecarboxamide compound invented and globally developed by Ishihara Sangyo Kaisha, Ltd.; and is characterized by its inhibiting effects on sap-sucking behavior of aphids and other sucking insect pests at low application dose rate. We focused on its unique activity which provides some species with abnormal motions and behaviors, and investigated its mode of action by using the whole-cell patch clamp technique. Flonicamid inhibited whole-cell outward currents evoked by voltage steps in a primary culture of ganglion cells of Spodoptera litura. In the study with the transformant in which the fast-inactivating A-type potassium channel (Kv4.3) was introduced, flonicamid did not show any inhibitory effect. The inhibition of outward currents observed in the ganglion cells of S litura seemed to be due to an inhibition of outward currents through delayed-rectifier potassium channels, such as seizure (sei), ether-a-go-go (eag), shaker cognate b (shab), shaker cognate w (shaw) or slowpoke (slo). Abnormal behaviors and defects in courtship song have been reported in Drosophila with mutations in the eag and slo genes, respectively. An inhibition of sap-sucking, abnormal behaviors and mating disruption are also observed in insect pests treated with flonicamid (Aphididae, Cicadellidae, Cixiidae and Drosophila). Taken together, the effects of flonicamid are suggested to be associated with the inhibition of delayed-rectifier potassium channels.

Keywords:*flonicamid, whole-cell patch clamp, delayed-rectifier potassium channels*

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P4

Eradication of the solanaceous fruit fly, *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae), from Yonaguni Island, Okinawa, Japan

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The solanaceous fruit fly, Bactrocera latifrons (Hendel), is known as a serious pest of solanaceous crops. This is widely distributed throughout South and South-East Asia, and is locally distributed in Hawaii and Africa. In Japan, It was first detected on Yonaguni Island (2,888ha) located in the westernmost island of Japan in 1984. There is concern that B. latifrons could spread to the south islands of Japan with shipped host fruits and/or the movement of adult flies. Therefore, eradication project for B. latifrons using sterile insect technique (SIT) was urgently implemented in 2004. Firstly, protein bait and clean culture methods were applied to suppress the wild population before implementation of the SIT. Mass release of sterile flies was initiated in Sep. 2007. About 400,000 sterilized insects were marked with fluorescent powder dye and released throughout the Yonaguni Island every week. Yellow sticky trap surveys were conducted from Oct. 2004 to Mar. 2010, and host fruit-surveys were conducted until Mar. 2011 to evaluate the control methods. The last detection of wild B. latifrons was in Mar. 2009 in host fruits-surveys. After that, no wild flies were found from host fruits-surveys for two years, in which 331,708 fruits were collected from 1,295 points throughout the Yonaguni Island. In addition to our surveys, Japanese government confirmed the eradication by their surveys of host fruits (170,863 fruits from 367 points) from Apr. to May. 2011. Altogether, B. latifrons was successfully eradicated from Yonaguni Island.

Keywords:*sterile insect technique, protein bait application, suppression control, fruit-collecting survey, yellow sticky trap survey*

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P4

Do closely related plants play a role in enhancing problems caused by *Meligethes aeneus* Fab?

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The presence of other cruciferous plants is usually undesired in oilseed rape fields. In this study we carried out small size plot experiments in 2006?2008 to test whether Brassica juncea and Sinapis alba are more attractive to the pollen beetle (Meligethes aeneus), do they allure more of this pests to the oilseed rape fields thereby increasing the damage caused. Furthermore, we tested the suitability of Brassica rapa as a trap crop protecting Brassica napus. We also investigated the impact of host plants on the oviposition preferences of parasitoids attacking the pollen beetle. According to our results none of the tested plant species increased the pest infestation of oilseed rape. On the contrary, both host plant species even decreased the damage caused by pollen beetles because they lured beetles at the most damage-susceptible stage of oilseed rape plants. Additionally, the parasitism rate was the highest on B. juncea and did not significantly differ between S. alba and B. napus. Thus, we can assume that close relatives of oilseed rape can also support the presence of natural enemies of pests in the field. The parasitism rate of the pollen beetle was surprisingly high, it reached up to 64.7% on B. juncea. Additionally the species composition of parasitoids of M. aeneus differed among plant species.

Keywords:*Brassica napus, Meligethes aeneus, B. rapa, B. juncea, Sinapis alba, parasitoids, attractiveness of host plants*

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P4

Fatal attractiveness? Host plant may kill its enemy's offspring

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Manipulation with attractiveness of host plants to insect pests is a useful tool in ecologically sustainable and economically viable integrated crop management. There is a growing interest in the value of enhanced agro-ecosystem diversity and "push-pull" strategies for suppression of insect pests. Plants that are suitable for feeding and oviposition but do not offer safe environment for development are called dead-end trap-crops. We compared oviposition activity and larval survival of Meligethes aeneus on the buds of Brassica napus, B. rapa, B. nigra, B. juncea, Eruca sativa, Raphanus sativus and Sinapis alba to find a potential trap-crop for oilseed rape. The number of M. aeneus eggs in green buds (BBCH 51?53) was lower on S. alba than on all other plants. The number of larvae in yellow buds (BBCH 59) on B. napus, B. nigra, B. juncea and R. sativus was greater than on B. rapa, E. sativa and S. alba. Brassica rapa was more attractive than E. sativa and S. alba but less than B. napus and B. nigra. Dead larvae were found only on E. sativa and R. sativus. The number of dead larvae on E. sativa was lower than number of alive larvae. The ratio of dead and alive larvae on R. sativus was almost equal, the average mortality was 46%. In conclusion, R. sativus had similar attractiveness as B. napus as an oviposition site for M. aeneus than oilseed rape, but perform as dead-end trap-crop because almost half of the larvae could not survive.

Keywords:*Meligethes aeneus, dead-end trap-crop, Brassica napus, B. rapa, B. nigra, B. juncea, Eruca sativa, Raphanus sativus, Sinapis alba, oviposition*

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P4

BICOPOLL: Targeted precision biocontrol and enhanced pollination

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BICOPOLL and BICOPOLL-NET connect 12 research units from 10 European countries and autonomous regions into a concerted effort to develop and bring into practice improved methods of biocontrol and pollination services, using strawberry as the case study. BICOPOLL partners will use bees to (i) precision deliver biocontrol agents to the flowers of the target crops to provide control of diseases, (ii) improve the pollination of horticultural crops. BICOPOLL will provide a pan-European case study on protecting strawberry from the grey mould. Additionally, we will improve the efficiency of the entomovector technology via innovative research on bee management, manipulation of bee behavior, and on the plant-pathogen-vector-antagonist-system, and will investigate possibilities of expanding the use of the concept into other berry and fruit growing systems. This is a highly innovative approach to solving some of the most difficult disease problems in berry and fruit production. The entomovector approach represents the only significant breakthrough in sight for improving plant protection in organic cropping systems and also conventional growers will benefit. We investigate, exploit, and support the natural ecological functions of biocontrol and pollination, and enhance these via innovative management. The entomovector technology contributes to improved resource use and efficiency in production, and enhances local biodiversity. The main target groups are organic and conventional strawberry growers, other berry and fruit growers, as well as beekeepers, to whom technology and knowledge transfer will be implemented during the project via direct contacts and by demonstration trials. The projects will cover three growing seasons during 2012?2014.

Keywords:*Strawberry, bee, bumble bee,gray mold,entomovector technology*

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P4

The use of companion planting for the management of the large white butterfly (*Pieris brassicae*) on white cabbage (*Brassicae oleracea* var capitata)

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Large white butterfly (Lepidoptera: Pieridae) (LWB) is a noxious pest of Brassicaceous plants from Europe to Asia, their feeding on foliage can significantly reduce crop value. LWB has developed resistance against some insecticides therefore a new approach is needed to prevent widely spread insecticide resistant populations conjointly with negative effects of insecticides. LWB has several natural enemies; the most efficient ones are parasitoids. The larval endoparasitoid Cotesia glomerata can infest 90% of pests. As vegetation diversity and density can influence the abundance of pests and natural enemies, companion planting with different flowering plants were used to study their effect on LWB and its parasitoid, C. glomerata. The small scale experiment was carried out in Latin square design, companion plants used were Agastache J. Clayton ex Gronov, Lobularia maritima L., Anethum graveolens L., the control treatment was without companion plants. To estimate pest occurrence, all plants were inspected weekly to count all LWB larvae. For the estimation of parasitism percentage, 4th-5th instar caterpillars were collected from the field and incubated in the laboratory. Results indicated significant effect of companion plant species on the mean number of LWB larvae per plant. Parasitism level was 30% on A. graveolens, 23% on L. maritima, 21% on control and 17% on Agastache. The attractiveness of Lobularia maritima to LWB and C. glomerata reveal its suitability as a trap crop preventing pests from attacking the main crop and attracting parasitoids as their natural enemies to keep their abundance under the economical threshold level.

Keywords:*parasitism rate, biocontrol, Cotesia glomerata, Agastache J. Clayton ex Gronov, Lobularia maritima L., Anethum graveolens L.*

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P4

Wild hosts of spotted wing drosophila a new threat to berry crops in Florida, USA and elsewhere

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Florida, USA produced ca. 4,050 ha of strawberries and 1,500 ha of blueberries in 2011. Strawberries reach markets December through early March and blueberries March into May with little competition form other sources and command high prices. Drosophila suzukii (Diptera: Drosophilidae), an Asian pest of thin-skinned fruit, first was detected in the Americas in California fall 2008 and France, Spain, Italy and other European regions soon afterwards. Its first discovery in Florida came in August 2009. No commercially damaging infestations of berries have been reported there. More than 2 years trapping on the University of Florida Gulf Coast Research and Education Center (GCREC) beginning December 2009 detected flies only in summer, a commercial berry-free period there, when American black elderberry, Sambucus nigra subsp. canadensis fruited. However in January through March 2012 adults were captured there near fruiting strawberry fields but no damage occurred on the strawberries nor on the blueberry crop that followed March through April. Many local wild and cultivated landscape fruits have been identified available year round to sustain D. Suzukii. These include balsam apple (Momordica charantia), Barbados cherry (Malpighia emarginata), fig (Ficus carica), podocarpus (Podocarpus macrophyllis), fringe tree (Chionanthus virginicus), hog plum (Ximenia americana), orange jasmine (Murraya paniculata), Simpson's stopper (Myrcianthes fragrans), Florida privet (Forestiera segregata), mulberry (Morus rubra), and American black elderberry. Information has been developed to assist strawberry growers confronted with the fly.

Keywords:*Drosophila suzukii, Diptera, pests, hosts, management*

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P4

Biological fitness and feeding behavior of imidacloprid-resistant and -susceptible brown planthopper, *Nilaparvata lugens* (Stal) on different resistant rice varieties

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The brown planthopper, Nilaparvata lugens is an economically important rice insect in China. Application of resistant varieties is the most economic method for controlling N. lugens populations, but chemical control is still a major method for suppressing the insect. The experiment indicated that on susceptible variety TN1, population number tendency index (PNTI) of imidacloprid-resistant and-susceptible N. lugens were 55.33 and 105.57, respectively; Relative fitness of resistant population to susceptible population was 0.5241. On resistant rice variety IR36, PNTI was 5.67 and 1.8, respectively, and relative fitness was 3.15. We found that PNTI of resistant and susceptible N. lugens at the 2nd, 3rd and 4th generations were 25.25 and 14.58, 14.52 and 11.85, 42.27 and 54.63, respectively. Relative fitness was 1.7318, 1.2253 and 0.7738, respectively. These indicated that imidacloprid-resistant N. lugens had stronger adaptability and survival advantage on resistant rice variety. Further experiment found seven classic waveforms of N. lugens on different rice varieties were recorded during feeding. On TN1, imidacloprid-resistant population had higher probe frequency, shorter probe time and longer phloem sap ingestion periods, but had no significant difference between two populations. On IR36, resistant population had lower probe frequency, shorter probe periods and longer xylem sap ingestion periods, but no found phloem sap ingestion waveform in two populations. With the increase of rearing generation, phloem sap ingestion periods of two populations further prolonged, especially imidacloprid-resistant population. The study will further elucidate resistant mechanism of rice and outbreak mechanism of N. lugens population.

Keywords:*Nilaparvata lugens, rice varieties, imidacloprid, biological fitness, feeding behavior*

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P4

Soil-borne insect pests in DPR Korea: Identification of key targets and timing for control

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Soil-borne insect pests have become increasingly important in crop production in DPR Korea (DPRK), with crop losses of 30% not uncommon. Control of these pests in DPRK has been hampered by the inability to implement preventive control techniques, such as crop rotation, or deploy effective plant protection products. The development of a locally viable biological control strategy, using entomopathogenic nematodes, has been proposed as a means to address this problem. Unfortunately, this strategy has been hindered by the limited local knowledge on the identification of key insect pests and their life-cycles, knowledge essential for the effective targeting of the biocontrol strategy. To address this lack of information field surveys were implemented in DPRK and soil insects collected for identification. In addition, reviews of available literature were carried out in DPRK. However, locally available information has proved limited. To gain more in-depth information on soil-borne insect pests which may be a problem in DPRK and their life-cycles, a literature survey was carried out focusing on the cold temperate and cold zones of East Asia (i.e. northeastern China, far southeast of Russia, Republic of Korea and DPRK) utilising the vast, non-English language source material. Information gathered to date will be presented and its use as a potential tool to support the development and implementation of a biocontrol strategy in DPRK discussed. This project is supported by the DG EuropeAid - Development & Cooperation (DCI-FOOD/2010/231-297) and the region-wide review by the Swiss National Science Foundation (IZK0Z3_140239 / 1).

Keywords:*Soil-borne insect pests, identification, biological control*

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P4

Harmonic radar finders as insect monitoring devices

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The study and monitoring of a wide range of organisms, including insects, are benefitting from the development of harmonic radar technology. The adoption of the technology has proceeded despite limited information on detection and recovery rates or on relative impact of the additional electronic tag load on insect mobility. The objective of this Canadian project has been to use a custom-designed portable harmonic radar to quantify the relative impact of those factors. Results carried out on three different insect pests (the plum curculio, the western corn rootworm and the Colorado potato beetle) validate the method as long as tags are adapted to each species and the radar scanning procedure adapted to the vegetative architecture.

Keywords:*Harmonic radar, Monitoring, Colorado Potato Beetle, Western Corn Rootworm, Plum curculio*

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P4

Effect of sublethal insecticide exposure on individual variation in activity and prey capture in the jumping spider *Eris militaris* (Araneae: Salticidae)

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Studying the maintenance of individual variation in behaviour is becoming a major topic in ecology. This variation can be especially useful as a mean for species to cope with environmental changes. Agroecosystems are ideal to study such questions since they display wide ranges of human-induced disturbances (e.g., chemical spraying, habitat fragmentation). In this respect, chemical spraying is of particular interest since numerous pesticide compounds are known to induce behavioural modifications at sublethal doses. Yet, the effects of such exposure on the expression of individual variation are currently unknown. After exposing the jumping spider Eris militaris to dry insecticide residues for 24h, we determined how a sublethal dose of phosmet affected individual variation in activity and prey capture. All spiders were tested prior and after exposure to the insecticide. Repeatability of activity was highly affected by the insecticide treatment while prey capture was not. In addition, random regression analysis indicated that insecticide-exposed spiders had higher activity in the first minute of the test but their overall mean activity was lower compared to non-exposed spiders.

Keywords:*Arthropod personality, Behavior, Spider, Eris militaris*

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P4

Leafhopper biodiversity in Canadian vineyards and feeding behavior of three *Erythroneura* species

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Leafhoppers (Cicadellidae) are pests of many temperate crops, including grapevines. Ca. 18,000 specimens were collected in 80 commercial vineyards located in the provinces of British Columbia, Ontario and Quebec (Canada). We identified 54 genera and at least 110 different species associated with vineyards, among which 22 were predominant and represented more than 91% of leafhopper specimens. We observed variations in species composition across the years and the provinces. Erythroneura species are the most abundant leafhoppers collected in Canadian vineyards. Due to the ability of leafhoppers to transmit phytoplasmas on grapevines during plant feeding, we studied the behavior of three Erythroneura species by electropenetrography in order to identify the damage and fed plant tissues. Our results indicated that Erythroneura species are essentially mesophyll feeders. As phytoplasmas are generally considered to be restricted to phloem sap, this brings into question the Erythroneura species ability to vector phytoplasmas in grapevine.

Keywords:*Leafhoppers, vineyards, Erythroneura, Feeding behavior*

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P4

The study on the protein bait of *Bactrocera dorsalis* (Diptera: Tephritidae)

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The oriental fruit fly, Bactrocera dorsalis, is one of the most important quarantine pests in Asian countries. It can feed on up to 250 different types of fruits and vegetables, causing severe ecnomic loss. Currently, there is no ideal control program. To finally effective control on these pests, we have developed a new bait based on protein X exhibiting very significant luring effect to both females and males with luring rate of 39.33%. In lab bioassays, two different lure baits both based on protein X were used. The lure rate of both baits reached peak at 24 h, with the one composed of 5g brown sugar: 5g protein X: 3g ammonium acetate: 50mL water was 55%, while the other composed of 5g brown sugar:5g protein X:10 mL white vinegar was 43%. The latter was then applied in the field bioassays and showed the most effective trapping activity on the third day. Adding 0.5% methyleugenol and 0.5% dichlorvos EC into the protein bait increased the trapping activity and the poisonous effect.

Keywords:*Bactrocera dorsalis, protein bait, trapping activity*

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IPM

P4

Study on non-chemical control techniques of *Thrips flavidulus* (Thysanoptera: Thripidae)

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Thrips flavidulus has caused great damages to citrus, peach and banana etc. orchards in China. In this study, the functional and numerical responses for the adults of the predatory mites Amblyseius cucumeris, Amblyseius barkeri and Euseius nicholsi on Thrips flavidulus first-instar larvae were studied to evaluate their predation ability on T. flavidulus. The results showed that the functional response of adult A. cucumeris, A. barkeri and E. nicholsi could be described by the Holling II functional response type. The results suggested that predation ability of A. cucumeris was strongest to T. flavidulus among three predatory mites and prey density significantly influence reproductive ability of A. cucumeris and E. nicholsi, but not A. barkeri. Among 10 types of colors sticky cards named yellow、blue、deep-blue、white、red、green、purple、gray and pink in citrus orchards, the thrips had the strongest preference to the blue sticky card and the deep-blue sticky card, on which the thrip numbers existed a significant difference from those on the other eight colors sticky cards ($p<0.01$). The trapping techniques of blue sticky card were discussed. Effect of 16 different plant volatile compounds on the behavior of adult female thrips was tested with a Y-tube olfactometer. The results showed that T. flavidulus were attracted by the compound of benzenoids and monoterpenes isoprenoids, and same compound at different concentration has different action. The compound A and E have the significant attraction to thrips at concentration 10% and 1%.

Keywords:*Thrips flavidulus,numerical responses,predatory mites,colors sticky cards,plant volatile compounds*

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Behavioral response of the egg-larval parasitoid Ascogaster reticulata to tea leaves treated with host eggs or other host materials

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The relationship between herbivore-induced plants and parasitoids receives attention recently. Chemicals released by plants when they are attacked by herbivores are useful for natural enemies to find their hosts in tritrophic interactions. An interesting phenomenon is that some plant species can respond to the first step in a herbivore attack of laying eggs on host plants. Ascogaster reticulata Watanabe (Hymenoptera: Braconidae) is a solitary egg-larval endoparasitoid of several tortricid species, including Adoxophyes honmai. Host searching behavior(s) of A. reticulata for hosts on tea leaves can be stimulated by cues produced from tea leaves not only infested by A. honmai egg mass deposition without visually recognizable damage but also leaves treated with A. honmai reproductive organs. Egg masses of female A. honmai induced tea leaves to arrest A. reticulata to spend significantly more time on treated leaves than control leaves. Moreover, elicitors in the mated female reproductive organ of A. honmai also induced tea leaves to produce cues that arrested parasitoids 24-48 hr after treatment of the leaves. In contrast, the total time parasitoids spent on infested tea leaves at 1 hour was not significantly different when compared with the control. The concentration of the female reproductive organ homogenate was important for inducing tea leaves to produce cues because the time spent by parasitoids for searching on tea leaves treated with the original concentration(reproductive organ+10µl Ringer's solution) was significantly longer than lower concentrations. The specificity of the elicitors is discussed based on these results.

Keywords:*tritrophic interaction, host searching behavior, egg-larval endoparasitoid, elicitor*

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Pyrethroid resistance in melon thrips, *Thrips palmi* Karny

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The melon thrip, Thrips palmi Karny, is among the most destructive pests of vegetables in western Japan because of its development of insecticide resistance. To examine its resistance mechanisms against a pyrethroid, cypermethrin, we determined partial nucleotide sequences of the sodium channel gene and compared the deduced amino acid sequences among 11 strains of T. palmi showing different levels of resistance. All the 11 strains had an amino acid substitution T929I alone. The synergist, pyperonyl butoxide, showed synergistic effects of the susceptibility to cypermethrin in the most resistant and least resistant strains. There was no significant difference in enzymatic activities of carboxyl esterase and glutathione-S-transferase between both strains. These results suggest that both reduced sensitivity of the sodium channel caused by T929I and CYP450-mediated detoxification are involved in the pyrethroid resistance of T. palmi.

Keywords:*Thrips palmi, resistance, insecticide, pyrethroid, sodium channel,*

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P4

Management of strawberry blossom weevil and European tarnished plant bug in organic strawberry and raspberry using semiochemical traps

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The strawberry blossom weevil (Anthonomus rubi) and the European tarnished plant bug (Lygus rugulipennis) cause large (10 - >80%) losses in yield and quality in organically grown berries. A consortium with 6 European countries has been created to work on the management of those pests. The pheromones of A. rubi and L. rugulipennis have been characterized in England by NRI/EMR. For the attraction of A. rubi the importance of host plant volatiles in combination with the pheromones has also been documented. The natural semiochemical mechanisms of sexual attraction and host plant finding of A. rubi and L. rugulipennis will be further studied and exploited to develop effective semiochemical traps for their management through mass trapping. Attractive lures for these two species will then be combined into a single multitrap with the aim of managing two pests simultaneously in each crop. This will be one of the first approaches to pest management of non-lepidopteran insect pests of horticultural crops using semiochemicals in the EU, and probably the first to target multiple species from different insect orders. The project will be organized in the following work packages; 1) Chemical analysis of plant volatiles, 2) Pest insects in strawberry, 3) Pest insects in raspberry and 4) Trap design and lure development. The authors gratefully acknowledge the financial support for this project provided by the CORE Organic II Funding Bodies, being partners of the FP7 ERA-Net project, CORE Organic II (Coordination of European Transnational Research in Organic Food and Farming systems, project no. 249667).

Keywords:*pest insects, strawberry, raspberry, semiochemical traps*

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Mating disruption of *Planococcus kraunhiae* (Kuwana) (Hemiptera: Pseudococcidae) with its sex pheromone in Japanese persimmon orchards

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We demonstrated the control of the Japanese mealybug Planococcus kraunhiae (Kuwana) by the mating disruption with sex pheromone from 2008 to 2010 in Japanese persimmon orchards. A grey rubber septum (8mm outside diameter, West Pharmaceutical Co., Singapore) impregnated with three milligrams of the pheromone was used a dispenser. Dispensers were set in orchards at the end of April in each year. The treated quantity of the pheromone was 1g/a (2008-2010) and 0.1g/a (2009). The treated area was 2.3a to 4a. As a result, number of caught males to a pheromone trap in the 1g/a treated place was far fewer than numbers of them in the no-pheromone place through the persimmon growing season (April to October) in each year. The rate of ovipositing females, which were enveloped in a net with diet and exposed in each place during several days before copulation, in the 1g/a treated place were almost zero through the persimmon growing season in each year. The infested fruit rate in the 1g/a treated place was lower than the rate in the no-pheromone place at any time in each year. In the 0.1g/a treated place, the above three parameters were occasionally worse than them in the no-pheromone place after September. We thought that the mating disruption in the 0.1g/a treated place worked until the end of June at least. From the above, mating disruption of Planococcus kraunhiae with the 1g/a sex pheromone was successful throughout the persimmon growing season.

Keywords:*mealybug, Planococcus kraunhiae, mating disruption, sex pheromone, Japanese persimmon, grey rubber septum*

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Population genetics of *Bactrocera dorsalis* s.s. and *B. papayae* across Thai-Malay Peninsula

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Sterile Insect Technique (SIT) is an environmentally-friendly based method to control insect pest. This method is relied on mating between released sterile and wild insects resulting in a reducing of pest population size from generation to generation. However, some key pests are members of species complex and exist in the same area. If genetic isolation exists between members of complex, use of SIT to control one species might not effectively control another members. Otherwise, if genetic exchange is detected, releasing sterile insects of only one species is possible to suppress other members. In this study, we explore the population genetics of two invasive fruit fly pests, Bactrocera dorsalis s.s. and B. papayae, members of the Bactrocera dorsalis species complex. We have analyzed the data of 12 microsatellite loci (i.e., seven originated from B. dorsalis s.s. and the rest loci originated from B. papayae) from 17 populations sampled in sympatric/ allopatric areas across Thai-Malay Peninsula. The preliminary data showed that genetic variation of two species was similar. In addition, genetic exchange between two species was observed in some locations, both sympatric and allopatric areas. This result was also congruent with no significant correlation between genetic and geographic distances. Population genetic data implies potential use of one SIT to control two species in some areas of Thai-Malay Peninsula.

Keywords:*Bactrocera dorsalis complex, microsatellite, pest control, population genetics, gene flow*

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The SLU Centre for Biological Control: Generating new knowledge on sustainable application of biological control

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At the new Centre for Biological Control (CBC) we conduct fundamental and applied research on biological control (i.e. the use of living organisms to control populations of harmful organisms), which aims to strengthen the knowledge base and facilitate the development and implementation of new biocontrol products and approaches. The centre works with augmentation, conservation and classical approaches, with utilization of both microorganisms and invertebrate animals. Close cooperation with stakeholders, such as growers, industry, authorities and organisations is an integral part of CBC activities. The research at the centre is lead by five scientists: Margareta Hokeberg's research area concerns bacteria for biological control. Her current interest is how to use combinations of different biocontrol agents and combining biocontrol with other control measures for sustainable disease control. She is also the CBC director. Hanna Friberg works on fungi in biocontrol. She has focused on plant pathogenic fungi causing disease in agricultural crops - how they can be managed without chemical control and how different cultural practices influence their abundance and activity. Sebastian Hakansson's areas of interest are that of long-term stabilization of microorganisms and life in the dry state, anhydrobiosis. Mattias Jonsson is specialised in insects and arachnids for biocontrol. His research is mainly focused on conservation biological control of invertebrate pests in agroecosystems. Ingvar Sundh works with issues related to safety and regulatory measures. He focuses on strategies to determine that a biocontrol agent has no unacceptable adverse effects in humans, non-target organisms in agriculture and forestry, and the general environment.

Keywords:*research, stakeholder cooperation, microorganisms, invertebrates*

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P4

Exploring a mass-production with an artificial diet in the sweetpotato weevil *Cylas formicarius*

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Two weevil species, Cylas formicarius and Euscepes postfasciatus, are world-wide major pests of sweet potato. In Okinawa, Japan, we are challenging to eradicate them by the sterile insect technique. To release high proportion of sterile target pests into a field population, a mass-rearing method with an artificial diet is a basic requirement. Although the artificial diet and rearing method for E. postfasciatus had been established and executed in a mass-production facility, the ones for C. formicarius have not been established yet. This mainly results from lack of 1) suitable substrate inducing female oviposition and 2) a method of collecting their eggs that are brittle relative to E. postfasciatus eggs. In the present study, we struggled with these problems. Firstly, we prepared several artificial diets chiefly consisting of sweet potato powder and agar, and reared C. formicarius gravid females individually during 35 days. They survived with these diets only and consistently laid eggs (0.14 egg/d). This is the first report of C. formicarius oviposition on an artificial diet. Secondly, we examined change in egg-shell hardness after oviposition to seek timing of collecting the egg without damage. The egg dramatically hardened as developing and eventually became over threefold hard as E. postfasciatus eggs, which can be collected by simple methods, suggesting that 3 days or later old C. formicarius eggs should be collectable. Thus, we overcame the two problems on the first step in exploring the artificial diet for C. formicarius. We will discuss further possible difficulties in establishing the mass-production procedure.

Keywords:*sweetpotato, pest control, sterile insect technique, mass-production, artificial diet*

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P4

Insecticidal effect of Jatropha curcas oil on the main insect pests associated with cowpeas (*Vigna unguiculata*) in Niger

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Application of J. curcas oil at 5% and 7.5% concentrations significantly reduce the level of attack by aphids (Aphis craccivora) by 10 and 50% respectively compared to the control. Firstly, a 50% and 75% fall in the number of thrips (Megalurothrips sjostedti) and bugs (Anoplocnemis curvipes), respectively, can be observed under the same conditions. This treatment allowed to greatly increase yields compared to the untreated parcel. Secondly, we compared the insecticidal activity of two batches of oil conserved during 70 days in plastic containers, one exposed to light and the other kept in the dark. The insecticidal activity of 5,10 and 15% of J. curcas oil conserved during 70 days was evaluated in a field test on the main devastating pests of Cowpea (Vigna unguiculata ((L.) Walp) . There was no significant difference in insecticidal activity between the two modes of conservation of J. curcas oil with various concentrations. The concentration of 10% in all modes on conservation allowed a reduction of devastating, more than 80% of thrips, aphids, bug and 75% of Jassides compared to the untreated parcel. The concentrations of 15% give an insecticidal effect comparable to (deltamethrine) but induce symptoms of phyto- toxicity on the leaves of cowpea.

Keywords:*Jatropha curcas oil, insecticidal effect, insect pests, cowpea*

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Application of semiochemical releasers and intercropping to control aphid and related virus in East China

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Nowadays, the control of aphids - well known virus vectors, lies on the use of synthetic insecticides. The chemicals' harmful effects on the environment and on human health led to some research aiming at the development of new alternative methods that would be efficient for producers. Within this context of biological control against aphids and their virus transmission, semiochemical substances (pheromone and allelochemicals) and the intercropping are strategies increasingly studied. The properties of the semiochemical moleculles were used to implement 'push-pull' techniques to repel pests and attract beneficial insects. Intercropping was applied as an attractive strategy for managing pests and viruses through the release of allelochemicals or by associating plants in intercropping. In this study, three semiochemical substances namely (E)-β-farnesene (EBF), cis-3-hexenyl acetate, garlic extract and peas intercropping were separately tested. Yellow traps were used and complemented by visual observation in the potato field plots corresponding to each kind of treatments. The evaluation of the abundance and diversity of aphids was performed weekly by collecting insects in fields and indentifying in the laboratory. The presence of viruses (Potato virus Y (PVY) and Potato Leaf Roll Virus (PLRV)) and the proportion of infected plants in each plot were evaluated by enzyme-linked immunosorbent assays (ELISA). Between the different treatments, the cultural association presented higher repulsive effects than the released semiochemical moleculles. Investigated strategies to control aphid and related virus were discussed in terms of efficacy and perspectives of application in larger scale for sustainable crop production.

Keywords:*Aphid, Integrated Pest Management, Biological control*

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Mating disruption for control of codling moth, *Cydia pomonella*, in commercial apple orchards in Quebec, Canada

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The codling moth, Cydia pomonella L. is a key pest of many fruits and nuts throughout the world. In the last 15 years, damage from this pest has been continuously increasing in all major apple regions of Quebec resulting in increasing number of annual sprays of insecticide needed for adequate control. In order to reduce the environmental impact and the risks for development of insecticide resistance, a decrease in pesticide use is desirable. Mating disruption (MD) has shown promise as a preventive tool to manage codling moth populations in many parts of the world. The objective of this project was to evaluate the efficacy of Isomate® CM-OFM TT for the management of codling moth under Quebec conditions in three apple orchards with different codling moth pressure. In each orchard, plot with MD (> 4 ha) was paired with a control plot treated with conventional insecticides alone. In order to reduce pest populations to levels more easily manageable, the standard spray programs were maintained to supplement control for the first year in MD-treated plots. Under moderate and high codling moth density, respectively 159 and 218 cumulative moth captures, four insecticide sprays were required to control codling moth and adequate control was achieved in both plots, with and without mating disruption (< 1,0 %). Under low codling moth density (53 captures), no insecticide treatment was done to control codling moth but high levels of damage (6,0 %) was observed in plot without MD while damage was near zero in MD-treated plot.

Keywords:*IPM, alternative control*

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Phenotypic and genetic diversity of rice varieties and relation to attack of *Diatraea saccharalis*

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The response of 34 accessions of rice (Oryza sativa L.) to infestation of stem-borer (Diatraea saccharalis Fabr.) was determined and characterized genotypically and phenotypically. Susceptibility to D. saccharalis was determined infesting plants with neonate stalk borer larvae (20 larvae/hill). The experimental design was a randomized complete block with four replications. Each plot was represented by a clump of ten plants. At the time of infestations plants were confined in cylinders (measuring 0,2m in high and 0,25m in diameter). Thirty five days after infestations plants were cut at the soil level, and brought to laboratory. All tillers were carefully examined looking for sign of attack, and presence of larvae. External and internal diameters of the stem and weight the surviving larva also were determined. For molecular analysis of rice varieties, 24 microsatellite markers were used. Highly significant positive correlations were recorded between survivorship of larvae and external and internal diameters of the stem. Molecular analysis of the rice genotypes in which 24 SSR loci were used, revealed a total of 243 polymorphic alleles with an average of 10.2 alleles per locus. Cluster analysis performed with genotypic data showed a significant genetic divergence among the genotypes of rice and a strait relationship between the phenotypic data that differentiated the genotypes more resistant to D. saccharalis. This study showed that there is a complete correspondence between the groups formed by these clustering methods under the two aspects analyzed (phenotypic and molecular).

Keywords:*plant resistance, rice genotypes, molecular biology*

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Pathogenicity test of the fungus *Aspergillus clavatus* on aphid *Acyrthosiphon pisum* (Aphididae)

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Pea aphid is a pest of many cultivated and wild plants, but also a vector of several viral diseases. To control this pest, the most widely used methods are physical, chemical and more recently an integrated approch that includes biological control. With the use of pathogenic agents against insects, the use of entomopathogenic fungi is one of the most promising. The present study demonstrated the possibility of using an entomopathogenic fungus Aspergillus clavatus against aphids. In laboratory conditions (8/16 photoperiod, average temperature 25oC), the insects were in contact with different concentrations ranging from 102 to 10 4 spores/cm2 deposited on filter paper in Petri dishes, or applied directly to young plants with doses ranging from 104 to 106 spores / ml. In 24 hours, mortality was 0 to 31.5% in Petri dishes. For treatment plants, the cumulative mortality in 5 days was 55 to 79%. Microscopic observations showed that the aphids were infected by contact and fungus has a mycosis effect. From these preliminary results, investigations should be made to study the action of the fungus on the reproduction of aphids. Therefore, A. clavatus could be introduced along with other fungi found in the literature as a biological control agent against aphids.

Keywords:*Aphid, Aspergillus clavatus, Biological control*

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Damage levels of two stink bugs (Heteroptera: Pentatomidae) that feed on rice stems in Brazil

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Tibraca limbativentris and Glyphepomis spinosa are the only stink bugs that have been feeding on rice stem in Brazil. Field and greenhouse studies were conducted from 2007 to 2012 in Santo Antonio de Goias, GO, Brazil, to determine rice yield-loss due to these two species of stink bugs. A randomized block design was used with four replications. Infestations with T. limbativentris were made with different insect densities at the beginning tillering, maximum tillering, and booting. The yield was regressed in function of number of stink bugs/unit of area, and the slope of the linear regression was used as the DI (yield loss/insect density) variable in economic injury level (EIL) calculations. In addition, experiments were conducted with one couple of G. spinosa per pot with rice plants. These adults remained on plants since infestation until the removal in the final of feeding time stipulated by the nine treatments. Injury of both stink bugs negatively affected most of yield components; however, damage imposed at vegetative phase was more severe than at the reproductive phase. Overall, EILs for T. limbativentris depended on plant stage: 0.5 adult/m2 in the maximum tillering and 1.0 adult/m2 in the booting. In conclusion, G. spinosa can reduce the rice yield more than T. limbativentris.

Keywords:*rice stem stink bug, Oryza sativa, rice small black bug, feeding time*

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Assessment of synthetic chemicals for disruption of *Rhynchophorus ferrugineus* attraction in an urban environment

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The red palm weevil (RPW), Rhynchophorus ferrugineus Olivier (Coleoptera, Curculionidae), is currently one of the most severe pests of Canary palms in urban areas of Mediterranean countries and date palms in cultivated areas of the Middle East. RPW aggregation pheromone can be implemented for both monitoring and mass trapping however risks of RPW 'spillover' onto palms is of great concern. Aim of this work was to discover repellent semiochemicals for RPW population management in urban environments. For this purpose we conducted electroantennographic (EAG) screenings of 17 commercially available synthetic compounds, representing three groups of plant volatiles (isoprenoids, phenyl propanoid derivatives and fatty acid derivatives) known for their repellent effects toward insects. EAG bioassays results showed positive dose-dependent responses to 13 of the 17 synthetic chemicals on RPW antennae of both sexes. These tests were followed by trap-based screenings of EAG-active menthone, α-pinene methyl salicylate, 1-octen-3-ol and citronellol, singly and in combination, under urban conditions. These trapping experiments were conducted in the city of Palermo, Italy during 2010, 2011 and 2012 to test the behavioural effect on the RPW adults. Experiments were carried out by placing the tested chemicals inside the pheromone baited traps and assessing a possible reduction in the adult captures. In particular were tested: in 2010 α-pinene and methyl salicylate, in 2011 α-pinene and menthone, in 2012 α-pinene, 1-octen-3-ol and citronellol. The results of trap tests showed that some of the compound tested significantly reduce the number of RPW captures in the traps.

Keywords:*semiochemicals, menthone, α-pinene, methyl, 1-octen-3-ol, citronellol, EAG*

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Comparison of aphidofauna in Belgium and Mali and potential impact on potato virus dispersion

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Climate changes focusing on global warming and the consequences on insect distribution are often cited mainly for exotic pests Here, studies were carried out during three years (from 2009 to 2011) in potato fields, to determine the abundance and diversity of aphids (Homoptera, Aphididae), and potential impact of potato viruses dispersion both in Europe, particularly in Belgium, and in west Africa, in Mali. Aphid populations were recorded by visual observations on potato leaves and using yellow water-pan traps. The monitoring was made weekly in different locations. The identification of aphid species was operated in laboratory, Most of the identified species were not potato colonizers. Main aphid transmitted virus, namely Potato virus Y (PVY) and Potato leaf roll virus (PLRV) were assessed by enzyme-linked immunosorbent assays (ELISA) technique. More than 30 species of aphids were identified in Belgium, while 15 species in Mali, of which seven are common to both countries (Aphis fabae, Brevicoryne brassicae, Lypaphis erysimi, Myzus persicae, Rhopalosiphum insertum, Rhopalosiphum padi and Tetraneura sp.) Major aphid species transmiting potato viruses were Myzus persicae, Aphis gossypii(from Mali), Macrosiphum euphorbiae and Aulacorthum solani (from Belgium). PVY and PLRV were variably occurring in Mali and Belgium, PVY being the most abundant. These resluts were performed as a step to monitor and apply some suitable strategies to manage potato aphids and related viruses.

Keywords:*Aphids, Potato virus disease, potato, vector, Mali, Belgium*

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Preliminary study of hydroxymethylfurfural (HMF) presence in syrup to feed bees in Belgium

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In 2009-2010 in Belgium, abnormal losses of colonies of bees were observed. Later analyses showed that some of these colonies had been fed during winter with syrup of inverted beet sugar which presented a high concentration of hydroxymethylfurfural (HMF). Several studies confirmed a toxic effect of the HMF on the health of the bee. At the moment, the absence of toxicological data of reference does not allow to establish a limit of definitive action susceptible to stop the problem. Preliminary study on HMF in syrups (for wintering or stimulation) to feed bees has been realised in order to point out HMF formation. We have compared several home-made syrups and commercial syrup often used by beekeepers in Belgium. Several measurements have been performed on all syrups stored at three different temperatures: pH, sugars composition, and HMF content. The results showed that most of the analyzed syrups contained mainly saccharose, had pH around 7 and a very little HMF content (< 5ppm). More interesting, commercial syrup and home-made syrup of water and saccharose mixed with pollen mainly contained fructose and glucose, and had acid pH around 3.5. Thus they contained the highest HMF content as well. These results highlighted the importance to standardize the production of syrup for beekeepers and especially for commercial producers that use directly fructose and glucose in their mixture.

Keywords:*bee, pesticide, residue, toxicology*

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Invasive exotic plant species in the Southern Appalachians (USA): Potential targets for biological control

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Invasive exotic plant species pose a risk to agriculture, forestry, natural areas, and urban habitats throughout the world. Numerous species are well established in the southern Appalachians in the United States, where they are serious economical and environmental concerns. The threat of some invasive plant species, such as kudzu (Pueraria montana var. lobata), multiflora rose (Rosa multiflora), and musk thistle (Carduus nutans), is well documented, and these species are well recognized by growers and landowners. Management programs, primarily focused on chemical, mechanical and cultural controls, have been developed to limit the spread of these exotic plant species, and biological control is a major component of integrated pest management of musk thistle. However, the threat of other species, such as Canada thistle (Cirsium arvense), purple loosestrife (Lythrum salicaria), and spotted knapweed (Centaurea stoebe L. subsp. micranthos), in the southern Appalachians is not as well known, and growers and landowners are not as familiar with these exotic species. In most cases, distribution of these invasive plant species has not been clearly defined. In addition, the diversity of native and exotic insect herbivores that utilize many of these introduced plant species has not been investigated. Biological control will be explored as a management tactic against selected exotic weeds based upon the 'best fit' for the area's climate and geography. This poster details selected invasive exotic plant species established in the southern Appalachians and examines the potential for the integration of biological control into pest management programs directed against them.

Keywords:*biological control, weeds, natural enemies, invasive species, exotic species*

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Status of the codling moth and the oriental fruit moth populations in apple orchards of Quebec, Canada

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In the last decade, population densities of the codling moth, Cydia pomonella (L.), have increased in most apple growing areas of Quebec, Canada. A second species of internal Lepidoptera, the oriental fruit moth, Grapholita molesta (Busck), has been observed for the first time in 2002 in the same area. Catches records from this time indicate that the distribution areas of these two apple pests are gradually expanding. Beside insect resistance and climate changes, we hypothesized that this growth could originate from (1) an increase in the numbers of weakly managed apple orchards including abandoned orchards, and (2) an increase of imported apples. To determine which of these two potential infestation sources could be involved, moth catches were followed weekly from the beginning of the adult flight to harvest, during two years, in the three most important apple growing areas of Quebec, Canada. Trap counts were compared from (1) weakly managed apple orchards; (2) monitored apple orchards; (3) unmonitored apple orchards and (4) storage sites of imported bins. Adult catches from these sites did not indicate a clear pattern in terms of infestation sources of these two internal Lepidoptera. In each apple region, the lowest number of codling moth and oriental fruit moth adult catches were observed from storage sites of imported bins. However, the rejection of our second hypothesis should be confirmed from the observation of individually wooded bins in order to detect newly emerged adults from overwintering cocoons.

Keywords:*apple pests*

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Prevalence of phytoplasma diseases in Canadian vineyards

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Leafhoppers are pests that can affect grapevines, notably by transmitting phytoplasmas. A survey of phytoplasma diseases was conducted in Canadian vineyards between 2006 and 2011. Leafhoppers and plants were sampled each year and phytoplasmas were identified using PCR and DNA sequencing. Phytoplasma DNA was detected in 40 cicadellid species. Several grapevine cultivars were also detected phytoplasma-positive. Disease incidence varied with the year, cultivar and province. Phytoplasma strains belonging to the Aster Yellow and Ash Yellow groups were detected.

Keywords:*vineyard, phytoplasma disease incidence*

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Insights into the potential of classical biological control in a changing climate

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Climate is the dominant force determining the distribution and abundance of most insects. There has been considerable concern in recent years about climatic warming caused by human activities and the effects of these changes on agriculture in North America. Warming conditions are likely to affect insect populations, both pest and beneficial species. Given that the magnitude of predicted temperature change associated with climate change is beyond the historical experience of modern agriculture, bioclimate models offer insights into the impacts of climate change and the issues facing development of future IPM strategies. The objective of the study was to develop bioclimatic models to predict potential range and relative abundance of a parasitoid P. digoneutis and its host Lygus spp. and to use the models to develop a better understanding of how climate affects the status of biological control efforts to manage Lygus populations in North America. The results of the study showed that range and distribution of the parasitoid and its host were positively and negatively affected based on specific combinations of temperature and precipitation. Sensitivity analysis indicated that P. digoneutis will continue to be successful even with the warmer temperatures predicted in future climates. In conclusion, the study provided insights that offered increased success for establishment of a classical biological control agent for the management of an insect pest. Adaptation strategies, such as classical biological control, can be employed to mitigate the effects of a changing climate.

Keywords:*classical biological control, changing climate, Lygus, P. digoneutis*

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Plant physiological disruption preceding hopperburn by a leafhopper

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Hopperburn is one of the defining symptoms of plant injury by a number of hemipteran pest species. Research on potato leafhopper, Empoasca fabae, has demonstrated physiological disruption of gas exchange processes (e.g., photosynthesis, transpiration) well before the appearance of hopperburn symptoms on their host plants, notably alfalfa, Medicago sativa. Here, we report on a series of experiments that demonstrate disruption of gas exchange processes and that help to elucidate the mechanism of injury. First, significant declines in photosynthesis and transpiration occur within 12 hours of the initiation of feeding, reaching an average decline of 30% and 32% of controls, respectively, by 24 hours. Second, light assimilation curve analysis demonstrated that 24 hour exposure to feeding causes a significant 25% decline in the light-saturated maximum rate of photosynthesis, without affecting the rate of respiration. Third, assimiliation-internal carbon dioxide analysis demonstrated that 24 hour exposure leads to variable but no significant effect on the maximum rate of carboxylation by Rubisco, the PAR-saturated rate of electron transport, and the rate of triose phosphate utilization. This final result is counter to the suggestion that this species causes end-product inhibition of photosynthesis, and instead suggests that stomatal behavior is the cause for reductions in gas exchange processes in injured plants. Our results differ from those with other hopperburning hemipterans, suggesting differences in mechanisms of injury among hopperburning species.

Keywords:*hopperburn, physiological disruption, photosynthesis, transpiration, potato leafhopper, Empoasca fabae*

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Volatile organic compounds of teak and behavioral responses of termites to teak volatiles

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Termites are the most destructive insect pests of wood. However, in Thailand teak (Tectona grandis) has been a popular constructing material for centuries because it is a well-known termite-resistant timber. In this research, we aimed to investigate the behavior of termites towards teak extract. Teak sawdust was extracted using dichloromethane and methanol as solvent. Then volatile organic compounds (VOCs) released directly from sawdust and from the extract were analysed using SPME-GC-MS. In addition, the chemical compositions of the extract were analyzed by LC-MS. VOCs released by teak sawdust belong to several chemical groups, i.e. alcohols, aldehydes, ketones, hydrocarbons, esters, and terpenes. There were 12 VOCs detected in headspace of teak sawdust and 33 VOCs identified in the extract. These compounds were, for example, 3-methyl-2-butenal, octane, nonane, α-pinene, β-pinene, 6-methyl-5-hepten-2-one, decane, hexyl acetate, limonene, undecane, and nonanal. Interestingly, some VOCs detected in sawdust headspace were not found in the extract. 2-Hydroxymethyl anthraquinone was found in the extract. This compound is a biologically active substance. The behavioral responses of termites to the characterized teak VOCs will be further described and discussed.

Keywords:*insect repellant, pest management, environmental pest control*

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Coordinated monitoring, forecasting and risk warning systems for field crop insects in western Canada

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The production area for field crops in Canada is extensive; the Prairie Ecoregion alone contains approximately 30 million hectares of cropland. Insect pest outbreaks are periodic/cyclical in nature. Production risks from insect pest outbreaks can be minimized through a coordinated monitoring program that provides decision-support to the agriculture industry in a timely manner. Also, monitoring of beneficial organisms is critical to their conservation and to the development of reduced-risk strategies for management of pest species. Climate change, new agronomic practices and new crops affect pest populations and diversity of beneficial arthropods. Knowledge and information on pest status, occurrence, forecasting tools, risk assessment analyses, and other outputs are made available through a number of vehicles including databases, models, software, technology transfer reports and publications. Wind trajectory analysis for air parcels that may carry insect pests and plant pathogens are also utilized to assess the potential for pest movement over hundreds of kilometers. Using the data collected, timely risk warnings, provided as regional risk maps and accompanied with interpretive text, are produced in a format useful to the industry (electronically, field days, producer meetings).

Keywords:*monitoring, forecasting, risk warning systems*

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Global effort to maintain susceptibility of the ryanodine receptor modulators and other insecticide modes of action: Efforts of the IRAC International Diamide (Group 28) Working Group

Companys Veronica[1], John T. Andaloro[2], Luis Teixeira[3], Isaac Billy Annan[4], Robert Senn[5], Russell Slater[6], Anna Armstrong[7], Ralf Nauen[8]

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Diamides (IRAC MoA Group 28) are a new class of insecticidal chemistry with a novel mode of action (ryanodine receptor modulator) that disrupt calcium movement in muscle cells resulting in rapid paralysis and feeding cessation. Flubendiamide which is a phthalic diamide was commercialized in approximately 2007 followed by registrations of chlorantraniliprole, an anthranilic diamide, a few years later. The diamides are highly effective against Lepidopteran pests and control insects resistant to other modes of action, thus creating the potential for over-dependency by growers. To maintain susceptibility of high risk insect pests to diamide products the IRAC International Diamide Working Group was created in 2008. They developed global IRM strategies for adaptation and implementation at the local level by country diamide IRM teams. The country IRM teams currently are comprised of IRAC company representatives and local influencers from academia, private industry, and extension. As of 2011, approximately 20 country IRM teams were created focusing on a few key insect pests. Additionally, the country teams are developing MoA communications and educational plans for influencers (trainers) and growers who will implement the IRM rotational strategies. The progress and accomplishments of these teams vary as some have been established for over two years while others are just being formed. Urgency to implement IRM strategies is necessary since Plutella xylostella resistance to diamide insecticides has already occurred in Thailand, Philippines, and Taiwan.

Keywords:*diamides, ryanodine receptor modulator, calcium channel, muscle cells, IRAC, IRM*

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Features and attributes of DuPont™ Cyazypyr™ (DPX-HGW86, Cyantraniliprole): A New Anthranilic Diamide Insecticide

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DuPont™ Cyazypyr™ (DPX-HGW86, cyantraniliprole), is the second anthranilic diamide insecticide (IRAC Group 28), to be commercialized but the first to demonstrate control of a cross-spectrum of different species and life stages of important insect pests in agriculture, seed treatment, and home and urban pest control sectors. The insect groups controlled include chewing pests (such as Lepidoptera, Dipteran leafminers and flies, beetles, weevils and sawflies), sucking pests (psyllids, whiteflies, leafhoppers, planthoppers and aphids), and rasping pests such as thrips. The mode of action is as a ryanodine receptor agonist causing rapid disruption of pest muscle function and resulting in rapid feeding cessation and other muscle-controlled insect activities. Due to the rapid feeding cessation of pest feeding plants are protected from direct damage during feeding and indirectly via reductions in pest-transmitted plant pathogens. Cyazypyr™ is efficacious through translaminar, foliar surface and soil application methods. It acts mainly through direct ingestion during pest feeding, so it has minimal impact on pest natural enemies. This allows products based on Cyazypyr™ to have outstanding selectivity to beneficials arthropods while still offering excellent pest control features. It also has low toxicity to mammals and other non-target vertebrates and invertebrates, excellent environmental fate profile, and also lacks cross-resistance with other classes of insecticides. These characteristics combine to make DuPont™ Cyazypyr™ an ideal complement to the integrated pest management (IPM) and insecticide resistance management (IRM) programs in integrated crop production systems.

Keywords:*DPX-HGW86, Cyazypyr™, anthranilic diamide, ryanodine, cross-spectrum, cyantraniliprole, translaminar*

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Different spectrum of insecticide resistance in the peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in populations from Beijing area, China

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The peach aphid Myzus persicae (Sulzer) is one of the most serious pest worldwide. High pesticide resistance level to lead to the difficulty on the control of this pest. Here, the pesticide resistance level to five frequently used pesticides were tested by bioassay on six field populations and a sensitive population, and the kdr resistance was detected using bi-PASA PCR method. Esterase and acetylcholinesterase activity was tested using traditional methods. The results show that both the six field populations, i.e. Tongzhou, Shunyi, Daxing, Haidian, Yanqing and Mentougou and the sensitive population developed resistance to beta-cypermethr. The proportion of resistant homozygote is higher than 90% in all studied populations except for Yanqing and Daxing; Mentougou, Tongzhou and Yanqing populations developed resistance to chlorpyrifos and imidacloprid with resistance ratio of 2.05-4.24 and 32.03-41.27, respectively; All of the field populations are sensitive to abamectin and emamectin benzoate (resistance ratio is 0.19-1.44), except for the Daxing population (resistance ratio is 3.45 and 2.82, respectively) and Haidian population to abamectin (resistance ratio is 2.66); There is significant positive correlation between the esterase activity and resistance ratio to chlorpyrifos and imidacloprid, while there is negative correlation between acetylcholinesterase activity and resistance ratio of chlorpyrifos and imidacloprid.

Keywords:*Myzus persicae (Sulzer), pesticide resistance, bioassay, enzymatic activity, knockdown resistance*

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Diversity and genetic differentiation of the whitefly *Bemisia tabaci* species complex in China based on mtDNA CO1 analysis

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The whitefly Bemisia tabaci are considered as a taxonomically complex that contained some destructive pests. Two of the most prevalent cryptic species are B. tabaci Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED). In an extensive field survey of the B. tabaci complex present throughout part of China from 2004 to 2007, we obtained 93 samples of B. tabaci from 22 provinces. We determined that these Chinese haplotypes included 2 invasive species (MEAM1 and MED), and 4 indigenous cryptic species (Asia II 1, Asia II 3, China 3 and Asia II 7) by sequencing mitochondrial cytochrome oxidose 1 gene (mtDNA CO1).Prior to 2007, MEAM1 was a dominant species in many provinces in China. By 2007, MED was dominant in 11 provinces. Both invasive and indigenous species were simultaneously found in some regions. Indigenous species of B. tabaci were found in six provinces in southern China. MED and MEAM1 have broad ranges of host plants, and indigenous species appeared to have much narrower host ranges. All Asia II 3 samples were found on cotton except one on aubergine. China 3 has more host plants than Asia II 3. Twelve samples of China 3 were collected from sweet potato, Japanese hop, squash and cotton.MED showed a lowest degree of similarity than the other species. The data indicate that both MEAM1and MED were rapidly established in China.

Keywords:*Bemisia tabaci, mtCO1, diversity*

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Life cycle (developmental) stages of *Hymenia recurvalis* (f.) (Lepidoptera: Pyralidae) on susceptible amaranthus species in Ibadan south-west Nigeria

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Aderolu, I.A. and Omooloye, A.A. University of Ibadan, Ibadan, Nigeria *Correspondence: adeisma@yahoo.com; +234-8035862166 The life cycle stages of the Beetworm moth, Hymenia recurvalis (F.) [Lepidoptera: Pyralidae], was studied on Amaranthus cruentus (susceptible host plant) and the adult fed with 5% honey solution (in cotton swab) under ambient conditions of 27±3oC and RH 75% in the screen house to identify resistance donor. Data were collected on morphometrics of immature stages (mm), developmental period (days) and number of instars and sex ratio. Oviposition commenced three days after mating The development of H. recurvalis on A. cruentus spanned 32.7days comprising egg which measured 0.57±0.254 mm long, 0.40±0.0435 mm wide and 0.24±0.0418mm in height. Eclosion (4.2±0.084days); 1st Instar (3.2±0.051days); 2nd Instar (2.3±0.041days); 3rd Instar (3.2±0.044days); 4th Instar (3.1±0.044days); 5th Instar (5.2±0.032days); 6th Instar (5.1±0.037days); Pre-pupa (2.01±0.043days) and Pupa (9.7±0.082days). The sex ratio was 1:1.09 in favour of female. Adult longevity: female (Unmated=15.3±0.56; mated=22.2±0.80) and male (Unmated=11.5±0.43; mated= 14.1±0.53) varied significantly. Average adult wingspans were 19.95±0.0.196 mm and 18.18±0.115 mm for female and male respectively.

Keywords:*Oviposition, Sex ratio, Eclosion*

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P4

Investigation of occurrence and spatial distribution of *Pseudaulacaspis pentagona*(Targioni-Tozzetti) on peach trees in greenhouse

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Pseudaulacaspis pentagona(Targioni-Tozzetti) is a kind of wide host range,feeding miscellaneous and reproductive ability pest,and has become a dangerous pest of fruit trees,garden flowers and economic forest in China for these years.With the development of peach trees in greenhouse in Beijing ,the occurrence and damage of P.pentagonaon had been reported. The occurrence,population distribution and spatial distribution pattern of P.pentagonaon on peach trees in greenhouse was investigated,which is basic work for effective prevention and control the pest.The results show that there are different damage among the peach species by P.pentagonaon. Which the number of female adults on flat peach orchard are significantly more than nectarine orchard,but there is no difference in number of male adults in both orchard. The population of the pests has a trend of aggregation in spatial distribution on peach trees in greenhouse,but no significant difference of damage is found between branches and bole.

Keywords:*Pseudaulacaspis pentagona(Targioni-Tozzetti), occurrence, spatial distribution, peach trees, greenhouse*

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Rapid expansion in geographic range of infestation of shiitake mushrooms by fungus gnat pest, *Neoempheria ferruginea* (Brunnetti) (Diptera: Mycetophilidae), in Japan

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The Shiitake mushrooms, one of the edible fungi in Japan, have mainly been cultivated with sawdust-based artificial media in an indoor facility over the last two decades. The amount of the mushrooms cultivated with this method represented more than 80% of the overall total in Japan. The infestation of larvae of a fungus gnat, Neoempheria ferruginea, which was first reported in 1992, has now increased to cover 19 of the 47 prefectures in Japan. The mechanism of this rapid expansion in geographic scope remains indeterminate. The larvae of the fly inhabit the surface of the media and the fruit bodies of the mushrooms, thus contaminating the larvae into the commodity causes damage of the mushroom production. This pest must be effectively controlled to prevent further expansion in the current distribution. To test our hypothesis: human activities such as transportation of the contaminated media to be linked to the rapid expansion; we examined to trace the geographical expansion with the phylogenetic analysis. We also conducted an experiment of the oviposition to the medium. We suggest that some populations expanded their distributions anthropologically because some remote localities shared the same haplotype of the mitochondrial DNA and the females laid eggs even on the surface of the plastic bags containing the artificial media. We also collected probably two different species of Neoempheria from the infested media based on both morphological and phylogenetic analyses. We suggest that suitable treatment of the media contaminated with the eggs is one of the effective in controlling measures.

Keywords:*edible fungi, pest management, Lentinula edodes, oviposition preference, genetic marker, geographic distribution*

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Specificity of pheromones traps for monitoring mealybugs in vineyards and pictoric key for males

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Mealybugs (Hemiptera: Pseudococcidae) are key pest in vineyards worldwide. Their cryptic habit make them hard to monitor, but recently sex pheromone of several mealybug species have been identified and synthesized, including the most common species in Chilean vineyards. This has allowed the use of pheromone baited traps for their monitoring. Fields trials were carried out setting out traps for five mealybug species (two reported for Chile and three not known to occur) for two seasons in nine vineyards. Male mealybugs caught in traps were counted and a proportion identified to species using morphological keys. The proportion of males caught in the corresponding trap was determined and male counts on traps were contrasted with species detected by visual counts on plants. The most common species found visually was P. viburni, with very few P. longispinus observed, which corresponded with the level of captures in traps. Pseudococcus viburni traps had on average ten times more males than P. longispinus traps (226 versus 20 males per trap per season), and only 1.2 males per trap per season were captured in the traps of the other species. On average for the two seasons 93.5% of identified males in P. viburni traps corresponded to this species, and 98% in P. longispinus traps. None of the males caught in the traps of species not known from Chile were from those species, representing only accidental catches. A pictorial key of male genitalia was developed. In conclusion pheromone traps showed a high level of specificity in the field.

Keywords:*pheromones, mealybugs, monitoring*

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P4

Seed treatment insecticide alternatives to manage soil insect pests of sugarbeet

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Soil-inhabiting insect pests frequently damage and cause severe economic loss in sugarbeet. Although conventional at-plant granular and liquid insecticide formulations have been used to manage these pests for decades, recently labeled insecticidal seed treatments have been widely adopted by producers in recent years. This research involved multiple field trials between 2004 and 2009 to compare insecticidal seed treatments with conventional insecticides for efficacy against natural infestations of the following: 1) sugarbeet root maggot, Tetanops myopaeformis Roder; 2) wireworms (Limonius spp.); and 3) subterranean Collembola (Onychiurus spp.). Excellent wireworm control was provided by the combination seed treatment Poncho Beta (clothianidin+betacyfluthrin applied at 60+8 g a.i./unit [100,000 seeds]), Cruiser 5FS (thiamethoxam; 60 g a.i./unit) seed treatment, and a conventional granular soil insecticide, Counter 15G (terbufos; 1.7 kg a.i./ha). Collembola control trials demonstrated that insecticidal seed treatments (i.e., Nipsit Inside [clothianidin; 60 g a.i./unit], Cruiser, and Poncho Beta) performed at similar levels to those of low and moderate rates (1 to 1.7 kg a.i./ha) of terbufos 15G. Seed treatment insecticides appear to be less likely than conventional insecticides to cause seedling phytotoxicity and associated yield losses in sugarbeet. The seed treatments we evaluated are likely to provide similar efficacy as moderate rates of currently labeled conventional soil insecticides for controlling wireworms and subterranean Collembola. A significant environmental benefit of seed treatment technology is that major (up to 95%) reductions in the amount of insecticide active ingredient are needed for adequate pest control in sugarbeet production.

Keywords:*Beta vulgaris, Limonius, Wireworm, Onychiurus, Collembola, springtail, clothianidin, thiamethoxam*

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P4

Comparative evaluation of VOCs emitted by *Achillea collina* infested by aphids and mechanically damaged

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Plants along the centuries evolved wide range of mechanisms to cope with biotic and abiotic stresses, including the emission of induced volatile organic compounds (VOCs), whose production is trigged by mechanical damage or pathogen infestation of plant tissues, providing them defensive tools (Arimura et al., 2005). This study focuses on VOCs emitted by Achillea collina Becker ex Rchb. as results of aphid infestation and mechanical damages. In particular it takes account of differences between compounds induced by the generalist Myzus persicae (Sulzer), the specialist Macrosiphoniella millefolii DeGeer aphid species infestation and two different types of mechanical injury (pin shoot and extended pressure). Headspace-Solid-Phase-Microexctraction and Gas-Chromatography/Mass-Spectrometry (HS-SPME-GC/MS) "in vivo" method was used to evaluate A. collina VOCs. We observe, in both aphids infestation a decrease of trans-2-hexenal associate with an increase of 2-hexen-1-ol and 1-hexanol, suggesting the activation of the methabolic pathway that leads to production of green leaf volatile compounds (Gatehouse, 2002). The same result was recorded in mechanically induced damages suggesting that in A. collina the production of these compounds is a non specific response to induced both by mechanically and aphids provoked injuries. Furthermore a decrease of some terpenes (β and γ-pinene) and the increase of others (α-phellandrene and isocaryophyllene) were recorded. The involvement of significantly varying compounds in tritrophic interaction mechanisms is also discussed.

Keywords:*Achillea collina, volatile organic compounds (VOCs), Myzus persicae (Sulzer), Macrosiphoniella millefolii DeGeer, HS-SPME-GC, MS*

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P4

Corn leaf aphid abundance in Cry3Bb1- and conventional-maize hybrids in South Dakota.

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A study to measure corn leaf aphid, Rhopalosiphum maidis (Fitch) abundance in corn rootworm, Diabrotica spp., resistant hybrids (Genuity® VT Triple PRO® corn) compared to conventional near isoline maize, Zea mays L., hybrids was conducted in eastern South Dakota during 2009 and 2010. The insect-resistant hybrid possessed a dual mode-of-action for above-ground insect protection in corn that is stacked with below-ground insect protection in addition its Roundup Ready® 2 herbicide technology. The Cry3Bb1 transgenic event was responsible for the resistance of this hybrid to corn rootworms. Plots were arranged in 9.2 meter square treatment areas (12 rows spaced 0.8 m apart) with 10 row buffers separations. To induce potential outbreaks of R. maidis, carbaryl insecticide was applied at 1.12 Kg[AI]/ha in half the plots on 10 July to eliminate beneficial arthropods and thus trigger later-season aphid resurgence. Aphid counts from whole-plant sampling were conducted by using a 2wk cycle from 10 August through 29 September. Aphid populations responded to the removal of predators and there were significantly more R. maidis found during sampling periods in treated conventional hybrid and Cry3Bb1 treatments. Additionally, we found greater aphid abundance in Cry3Bb1 plots over the near isoline irrespective of carbaryl treatments. The insect resistance hybrid was possibly more attractive to aphid colonization because they were unstressed from insect damage and withstood drought stress better than the near isoline.

Keywords:*Corn rootworm, Diabrotica, Maize, Rhopalosiphum maidis, aphid, insect resistance*

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Laboratory and field evaluation of *Isaria Fumosorosea* (wize) brown and smith against cotton pests

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The present study evaluated the role of entomopathogenic fungus, Isaria fumosorosea isolated from Alagar hill of Madurai dt, Tamil Nadu, India against Spodoptera litura in the laboratory as well as pests of cotton in the field. The results of the laboratory bioassay unveiled the susceptibility of S. litura to maximum of 80% reduction over control. The present study have recorded 3 (112±0.92 to 35±0.074) fold reduction of Aphids and 10 (11.13±0.86 to 1.6±0.002) fold reduction of Jassids in the field by I. fumosorosea. The probable reduction of bolls of cotton after spray has reduced drastically. It supports the suitability of virulent Ifr for the field cotton pest management.

Keywords:*Bioassay, Spodoptera litura, Isaria fumosorosea, Aphids, Jassids*

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Development and Reproduction of *Ophraella communa* (Coleoptera: Chrysomelidae) on *Ambrosia artemisiifolia* and *Parthenium hysterophorus* and its Potential for Biological Control

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Ragweed (Ambrosia artemisiifolia L.) and parthenium weed (Parthenium hysterophorus L.) seriously affect agriculture, human and animal health, as well as the environment. Both these plants are invasive weeds in Taiwan. Chrysomelids, scales, mealybugs, tussock moths, and leafminers have been found on both these weeds. Among these phytophagous insects, the ragweed beetle (Ophraella communa LeSage) is the most promising species for biological control. The temperature range for their development was 20 ~ 32℃, their egg incubation period ranged from 4 to 10 days, larval developmental time was from 9 to 15 days, and pupation period was 5 to 9 days. Under the same temperature, immature developmental times were similar when fed on ragweed or parthenium weed. The emergence rates and number of eggs laid per female were higher on ragweed than on parthenium weed. At 28℃, females on ragweed laid more than one thousand eggs, which is about twice the number of eggs laid on parthenium weed. Compared with parthenium weed, that ragweed was a better host for this beetle to survive and reproduce. Ragweed or parthenium control with this chrysomelid in restricted spaces was successful. When beetles were released on either of these weeds, the plants were seriously damaged within several weeks. But in area without other host plants, adults placed on ragweed plants disappeared shortly after release. Establishment of field population is the next major problem to be solved for further use of this natural enemy for biocontrol.

Keywords:*Ophraella communa, Ambrosia artemisiifolia, Parthenium hysterophorus, biocontrol*

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P4

Outcomes of intraguild interactions between two egg parasitoids in field and semi-field conditions

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Intraguild interactions between two egg parasitoids, Trissolcus basalis (Wollaston) and Ooencyrtus telenomicida (Vassiliev) exploring egg masses of the Southern Green Stink Bug Nezara viridula (L.) were investigated in field and semi-field conditions. Field trials were conducted in tomato fields by using sentinel and naturally laid egg masses in 2010 and 2011. Egg parasitoids monitoring, by sentinel egg masses placed weekly on plants during the tomato growing season and recollected one week later, showed that T. basalis occurs from June to October, whereas O. telenomicida occurs mainly in July and August. In the same fields, collections of naturally laid egg masses display different types of parasitized egg masses: only by T. basalis, only by O. telenomicida and by both species. The outcomes showed that T. basalis achieves a higher impact on the host population than O. telenomicida, even when multiparasitism occurs. Semi-field trials were conducted from July to September 2011, using pepper plants covered with mesh net in order to create small cages with five plants inside. In each cage, egg masses were placed on four plants located near to the cage corners and then T. basalis and O. telenomicida were released individually or simultaneously on the central plants. Egg masses were recollected after one week. The results showed that T. basalis achieves a high parasitization level when released alone but suffers competition by O. telenomicida when both species were simultaneously released.

Keywords:*biological control, competition, Nezara viridula, Trissolcus basalis, Ooencyrtus telenomicida*

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P4

Transmissibility of saffron infecting potyvirus in Iran by different aphid species

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Saffron (Crocus sativus L., Iridaceae) is the most expensive spice of the world and Iran, with 50000 ha saffron cultivation area, is the world's largest producer (> 90% of global production) of saffron. Infection of saffron plants with a potyvirus has been recently reported from Iran. Based on non-persistent transmission of potyviruses by aphid, this study was carried out to investigate transmissibility of this potyvirus by Aphis gossypii, A. fabae, Mayzus persicae and Acyrtosyphon pisum. Virus-free aphids were fasted for 2-4 h and then placed on infected saffron plants for an acquisition access period of 3-4 min. Afterwards, they were transferred in groups of five to the healthy saffron plants and Nicotiana benthamiana as positive control. Aphids were killed with an aphicide 24 h later and the plants transferred into an insect-proof glasshouse for 8 weeks. Detection of virus on inoculated plants was established by ACP-ELISA using potyvirus genus specific antibodies and reverse transcription PCR (RT-PCR) using potyvirus specific universal primer pairs at 14, 30 and 50 days after inoculation. The results showed that saffron infecting potyvirus was transmitted by A. faba into both saffron and N. benthamiana. The virus was not detected in saffron plants which were inoculated by other aphids, while it was detected in inoculated N. benthamiana plants. Therefore infected corms and A. faba are important factors of virus distribution in saffron fields.

Keywords:*Saffron, potyvirus, ACP-ELISA, RT-PCR, Aphis gossypii, Aphis fabae, Mayzus persicae, Acyrtosyphon pi*

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P4

Development and implementation of an area-wide management of Asian citrus psyllid (*Diaphorinacitri*Kuwayama) in Texas

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The Asian citrus psyllid, DiaphorinacitriKuwayama, is one of the known vectors of the currently incurable and deadly citrus greening disease or Huanglongbing (HLB). The insect is an introduced invasive species in the United States, and together with its vectored greening disease, D.citri is threatening the profitability and sustainability of the U.S. citrus industry. Due to its high mobility and high reproductive potential, this disease vector is very difficult to manage using local grower-level control approaches. This study presents strategies for the development and implementation of a pro-active area-wide management program for D. citri in Texas. The goals were to evaluate the importance of a dormant spray that targets overwintering adult psyllid populations and to demonstrate the viability of a regional control program over a local spray strategy. In a year-long-field study throughout 2008, a dormant spray application over a regional level provided effective control of D. citrifor 4 months, twice as long as with the local dormant spray approach. Throughout the year, the regional control program significantly reduced D. citripopulations over a large area thus preventing rapid re-infestation of groves by the pest, while only short term population suppressions were obtained with the local spray program. Grower participatory trials conducted in 2009 and 2010 showed that although coordination of the area-wide spray program is feasible during the dormant season its implementation during the active growing season from March to September is very difficult in Texas.

Keywords:*citrus psyllid*

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P4

Selection and characterization of a *Spodoptera frugiperda* strain resistant to transgenic maize expressing a *Bacillus thuringiensis* protein

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Availability of laboratory-selected insect strains is important to assess the potential for resistance development in the field and to improve resistance management strategies to transgenic plants. Transgenic maize event TC1507, expressing the Cry1F protein from Bacillus thuringiensis, has been used effectively for management of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Brazil. A strain of S. frugiperda, obtained from field collections in Minas Gerais state in 2010, was selected in the laboratory for resistance to Cry1F using leaves of TC1507 maize plants in two selection regimes. Chronic exposure of larvae to the event was more effective than gradual exposure in the selection of resistant S. frugiperda individuals. After only four generations of selection a strain with significant levels of resistance to Cry1F was obtained, as indicated by survival of insects recovered from Cry1F maize plants in the greenhouse. Importantly, selection for Cry1F resistance did not affect the survival of the selected strain in maize hybrids expressing Cry1Ab or the stack of Cry1Ab.105 and Cry2Ab2, indicating no cross-resistance between these toxins and Cry1F. Additionally, reciprocal crosses between control and the Cry1F-selected insects revealed that the resistance is autosomal and incompletely recessive, and there was no apparent fitness costs associated with the resistance. The results of this investigation have important practical implications for resistance management, which will be discussed in the poster. The availability of this resistant strain provides an opportunity to refine recommendations for resistance management strategies of S. frugiperda to transgenic maize expressing B. thuringiensis toxins.

Keywords:*fall armyworm, Cry1F, resistance management, inheritance, cross-resistance, Bt toxins*

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Assessment of *Harmonia yedoensis* Takizawa (Coleoptera: Coccinellidae) as a new agent for biocontrol

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In this study we try to reveal possibility for use of Harmonia yedoensis Takizawa in the biocontrol. H. yedoensis by several parameters of fitness, at comparison with Harmonia axyridis Pallas, was estimated. The total voracity of larvae H. yedoensis was greater than that of H. axyridis in all variations of the experiment. When using Myzus persicae and Sitobion avenae as prey, the voracity of H. yedoensis larvae was significantly greater than that of H. axyridis. Also, significant differences in developmental time of the immature stages were observed between tested species of ladybirds with S. avenae as prey; the time of development of larvae H. axyridis was shorter (10.2±0.18 days) than that for H. yedoensis (8.2±0.14 days). The survival rate of the immature stages was high for both species of ladybirds. But when using M. persicae as prey, the survival rate of pupae decreased by 30 % for H. yedoensis and by 15 % for H. axyridis compared with S. avenae. The first results of an assessment of H. yedoensis testify to the potential high fitness of this species for biocontrol. After some selection, H. yedoensis could be introduced in existing technologies of mass rearing of aphidophagus-ladybirds and could replace H. axyridis in the modern systems of plant protection.

Keywords:*Harmonia yedoensis, Harmonia axyridis, the fitness parameters, biocontrol*

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P4

Monitoring Pesticide Resistance of Two Spotted Mite, *Tetranychus urticae* in Rose Greenhouses in Korea

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Abstract Tetranychus urticae was collected from greenhouse roses to monitor the development of acaricide resistance. Dose-mortality lines were estimated on 16 regional populations with 13 acaricides. For each acaricide, LC50s of the populations were plotted to check normality. LC50s of eight acaricides showed normal distribution and five others did not. An index of Ln(recommended dose/LC50 for each acaricide) checked the development of resistance to populations. The index is based upon recommended dose to control a pest stage and empirical LC50 got from serial dilution range for each pesticide. We tried to categorize acaricides by index due levels of effectiveness to mites: tebufenpyrad, fenpyroximate, bifenthrin, and fenbutatin oxide as non-effective acaricides with less than index 1.0, chlorpyrifor+bifenthrin and milbemectin as alert level placed between 1.0 and 2.0, acequincyl as caution between 2.0 and 3.0, and abamectin, cyflumetofen, bifenazate, chlorfenapyr+fulacrypyrim and propargite as effective over 3.0. We also tested the similarity of acaricide actions for choosing effective acaricides against resistant mites and of populations for resistance management. We could make several acaricides groups: group1 including abamectin, cyflumetofen, and bifenazate; group 2 propargite and chlorpyrifos+bifenthrin; group 3 chlorfenapyr and acequinocy; and group 4 mibemectin, fenyroximate, and bifenthrin, by which we can suggest not to use acaricide within the same group to avoid the resistance development. Populations grouping would imply similar practices of acaricide use, so we can manage pesticide usage, effectively. Group A includes Gimhae 2, 3, 4, 5, Jincheon2, and Taean, and Group B includes Goyang, Gangjin1, Paju, Gangin2 and Namwon.

Keywords:*Resistance monitoring, Pesticide, Tetranychus urticae, Rose, Cluster analysis*

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P4

Larvicidal and repellent activity of Korean endemic *Thymus magnus* Nakai against the Asian tiger mosquito, *Aedes albopictus*

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The repellent and larvicidal activity of dagalet thyme, Thymus magnus Nakai was assessed against the Asian tiger mosquito, Aedes albopictus. Intact whole plants of T. magnus proved to have a repellent activity of 91.2% against female Ae. albopictus. The volatile compounds of T. magnus were determined by gas chromatography-mass spectrometry as follows: ?-terpinene (33.0%), thymol (29.9%), ?-bisabolene (8.9%), ?-cymene (8.3%), ?-terpinene (5.0%), myrcene (4.7%), ?-caryophyllene (4.0%), ?-thujene (2.7%), camphene (1.3%), carvacrol (1.2%), and ?-pinene (1.1%). Among these candidates, thymol exhibited complete (100%) repellent activity against the female Ae. albopictus, an effect that was confirmed through evaluating the electrophysiological response on the antenna of Ae. albopictus. Duration of effectiveness was produced by a binary 1:2 mixture of thymol and vanillin (0.05:0.1 ?L cm-2) that was found to be significantly more effective than thymol alone for a period of 90 min. In addition, thymol, ?-terpinene, and carvacrol showed high larvicidal activity on the third instar larvae.

Keywords:*Thymus magnus, Aedes albopictus, repellent activity, larvicidal activity*

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Within-tree Distribution of *Dialeurodes citri* (Homoptera: Aleyrodidae) Immature on Citrus Trees

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Citrus whitefly, Dialeurodes citri (Ashamed), is a minor pest of citrus in Jeju, South Korea. The occurrence of this pest is gradually increasing, especially in organic farming citrus orchards. Within-tree distribution of nymphs of D. citri on leaves of citrus, Citrus unshiu Marcow., trees were studied in orchard plots for application of entomopathogenic fungi, Aschersonia aleyrodis as biological control agent. Distribution of nymph stage depended on leaf position in a twig. The leaves with most abundance and low variation of citrus whiteflies were leaf 4th from a twig base. Taylor's power law provided better description of mean-variance relationship for the indices than Iwao's patchiness regression. The minimum sample size was estimated from the parameters of Taylor's power law for leaf 4th at precision levels of 0.20, 0.25 and 0.30. The sample size increased with improving precision level and decreased with increasing density of nymphs. The analysis of variance of two-stage sampling was used to estimate the number of sampled leaves per tree. The estimated variance decreased with increasing the number of sampled tree and sampled leaves a tree. The six leaves per tree was estimated as the optimum sampled leaves.

Keywords:*Dialeurodes citri, citrus, within-tree distribution, sampling*

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Occurrence Tendency of Major Pests in Korean Apple Orchards for Past 20 Years

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The annual occurrence of major pests and the damages by its were surveyed and analyzed at sixteen to thirty apple orchards belongs to four to eight major apple producing districts from 1999 to 2011. In mites, Tetranychus urticae showed the highest in orchard rates of occurrence as well as heavy population until 1990's, but the damaged orchards(%) was changed to Panonychus ulmi, even though the population density of Tetranychus urticae was still highly occurred. In aphids, Myzus malisuctus showed the difference by year, 0~38 % in orchard rates of occurrence and 0~2.5% in damaged leaf rates. But Aphis citricola showed higher in orchard rates of occurrence and showed big difference in population density according to year. In Lyonetiidae, Phyllonorycter ringoniella after 2000's has lowered pattern in the orchard rates of occurrence and damaged leaf rates compare to the beginning of 1990's. Lyonetia prunifoliella in the beginning of 1990's has showed pattern increased damaged leaf rates as sharply increased orchard rates of occurrence but decreased these rates after 2009. in the fruit moths, orchard rates of occurrence showed in 5~67% and damaged fruit rates in 0.01~3.1%, with difference by year. Since 1998, orchard rates of occurrence and damaged fruit rates of Grapholita molesta was showed higher continuously. In leafroller, orchard rates of occurrence and damaged fruit rates showed decreased patterns since on the beginning of 2000's. These population fluctuation on each major pests was compared to the annual weather (precipitation and mean temperature), areas of orchards and current status of agrochemical

Keywords:*Apple, Pests (mites, aphids, leaf miner, fruit moths, leaf roller), Population fluctuation, Damages*

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Control effect of mating disruption, Confuser R and Isomate KR to fruit moths in Korea apple orchards

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The control effect of Confuser R and Isomate KR, newly developed mating disruption to two fruit moths, Grapholita molesta and Carposina sasakii was assessed at the two organic apple orchards in Chilgok and Chungsong provinces, which was composed of four treatments; treatment of mating disruption Confuser R, treatment of mating disruption Isomate KR, no treatment of mating disruption and no treatment of synthetic pesticides(NTMD+NTSP), no treatment of mating disruption and classical treatment of synthetic pesticides(NTMD+CTSP). Annual monitoring using sex-pheromone traps for Grapholita molesta and Carposina sasakii in NTMD+NTSP fields were counted higher numbers as 457, 323 in Chilgok, respectively and 318, 30 in Cheongsong, respectively. Damaged fruit ratio by Grapholita molesta and Carposina sasakii before the harvest was recorded 14.3, 34.3% in Chilgok and 28.8, 10% in Cheongsong, respectively. Confusor R and Isomate KR treated orchard was exhibited the mating Inhibition ratio of Grapholita molesta adults over 99.8% in all Chilgok and 99.1 and 92.8% in Cheongsong areas. For Carposina sasakii, mating inhibition ratio was perfect. When treated Confusor R and Isomate KR, damaged fruit ratio was showed the control effect over 95% with no statistical difference to NTMD+CTSP. From above results, newly developed chemicals (Isomate KR) have the similar control efficacy with commercially registered chemicals (Confusor R) to two fruit moths

Keywords:*Confuser R, Isomate KR mating disruption, apple, fruit moths*

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Recent Occurrence Status of Three Major Species of Fruit Moths in Apple Orchards

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The study was conducted to investigate the population fluctuation on three major internal feeders, Grapholita molesta, Grapholita dimorpha, and Carposina sasakii, of six apple production regions of Korea from 2010 to 2011. The adult emergence of G. molesta, G. dimorpha and C. sasakii was examined by using pheromone traps. The population density of three species was ranked as follows: G. molesta > G. dimorpha > C. sasakii. The occurrence ratio of G. molesta, G. dimorpha and C. sasakii was 61.6, 28.3 and 10.1% in 2010, and 44.2, 40.6, 15.2% in 2011. The difference of occurrence ratio among was significant among apple orchards and between experimental years. The average number of G. molesta, G. dimorpha and C. sasakii caught on pheromone traps was 508.6(109 ~ 1,190), 233.2(16 ~ 797), and 83.4(1 ~ 255) in 2010, and 398.6(171 ~ 701), 366.3(186 ~ 758), and 137.0(14 ~ 468) in 2011. G. molesta and G. dimorpha adult was emerged from mid of April to late September and they have three or four generations per year. The first peak of overwintering G. molesta adult was showed on late April or early May. The first peak of overwintering G. dimorpha adult was later than that of G. molesta. C. sasakii has one or two generation per year.

Keywords:*apple, fruit moths(Grapholita molesta, Grapholita dimorpha, Carposina sasakii), sexpheromone trap,*

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Recent Occurrence Status of *Panonychus ulmi* and *Tetranychus urticae* in Apple Orchards

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The study was conducted to investigate the population fluctuation of two major spider mites, Panonychus ulmi (ERM) and Tetranychus urticae (TSSM) on 107~160 apple orchards of five apple production regions (Boeun, Yesan, Gunwi, Eusung and Bonghwa) of Korea from 2009 to 2011. The population density of TSSM was also examined on broad leaf weeds underneath the main apple trunk. The average occurrence rate of ERM and TSSM for three years was 17.0 (10.7 ~ 29.6) and 10.7(0.9 ~ 20.6)%. The average occurrence density of ERM and TSSM for three years was 8.1(1.6 ~ 26.7) and 4.3(0.01 ~ 12.0). The occurrence rate and density of ERM per year were presented as follows: 2010 > 2009 > 2011 and 2009 > 2010 > 2011. The occurrence rate and density of TSSM were ranked as follows: 2009 > 2011 > 2010. The occurrence rate of two spider mites per year was different among apple orchards. The population density of two species was highest on mid of May. The occurrence density and rate of TSSM on weeds for three years were 1.2 mites and 5.2%. The density of TSSM on weeds was lower than that of TSSM on the leaves of trees. The occurrence trend of TSSM on weeds was opposite to that on leaves of trees.

Keywords:*apple, spider mites (Panonychus ulmi, Tetranychus urticae),population fluctuation*

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Oviposition model of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

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Frankliniella occidentalis, a vector of tomato spotted wilt virus (TSWV), has spread nationwide with damage to vegetables and floricultural crops in South Korea. To develop oviposition model of this species, we investigated F. occidentalis adult females longevity and total fecundity from 13-20 individuals at constant temperatures (16, 19, 22, 25, 28, 31 and 35℃) and a photoperiod of 14:10 (L:D) h. The longevity was longest as 29.83±5.43 (mean ± SE) days at 16℃ and was shortest as 1.25±0.01 (mean ± SE) days at 35℃. Ovipositon did not occur at 35℃, and total number of eggs laid was fewest at 28℃. Temperature-dependent development was well described by Logan 6 model (r2=0.93), and were used to calculate the adult physiological age. The relationship between total fecundity and temperature was well described by the Taylor model (r2=0.80). Age-specific oviposition and survival rate were well explained by the three-parameter Weibull function (r2=0.87) and the sigmoid function (r2=0.89), respectively. F. occidentalis oviposition model was constructed by total fecundity model, age-specific oviposition rate model and age-specific survival rate model.

Keywords:*Frankliniella occidentalis, Ovipositon model, Temperature*

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P4

Enhanced repellency against *Aedes aegypti* by composition of plant essential oils with vanillin addition and its application

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Six plant essential oils, vanillin, and their mixtures were tested for repellent activities against a dengue virus vector mosquito Aedes aegypti (L.). These essential oils showing strong biological activities in fumigant toxicity test also gave repellent activities. Compared with 5% N, N-diethyl-3-methylbenzamide (DEET) of the six essential oil concentrations did not show repellent effects. However, a mixture of lemongrass oil, xanthoxylum oil, and vanillin (1:3:1, vol:vol:wt) showed that mixtures of essential oils with vanillin showed 270 min of complete protection time (CPT) compared with 247.5 min CPT with 15% DEET. The CPT depended on concentration of vanillin or on both factors. When we applied a mixture of lemongrass oil: xanthoxylum oil: vanillin (1:1:1, vol:vol:wt) to the Viscopearl formulation, or porous cellulose beads that provide gradual release of volatile compounds. Experiment showed 90% of repellency for 2 h. The insecticide and repellent behavioral approaches we drew upon in our current study insist that mixtures of vanillin and plant essential oil showed strong and durable repellency to the mosquito. Our study also indicates that personal repellents using mixtures of plant essential oils with vanillin were possibility applicable to spatial repellents and viable commercial product.

Keywords:*Aedes aegypti, mosquito, plant essential oils, vanillin, repellency*

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P4

The effect of exposure to a pulsed yellow light-emitting diode (LED) on the adult flight behavior of the cotton ballworm, *Helicoverpa armigera*

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Yellow fluorescent light irradiation has been commonly used for pest control of noctuid moths in various cultivated fields in Japan, but this method cannot be used in chrysanthemum fields because chrysanthemums produce buds only under the short-day condition. Therefore, an experiment was carried out to clarify the pulse conditions of a yellow LED required to control noctuid moths without suppressing the blooming of chrysanthemum. We used a lemon-yellow LED (central wavelength: 571nm) of similar wavelength to yellow fluorescent light, with intensity of 20mW/m² and pulsed-LED light period of 20ms. We observed the flight activity of adults of Helicoverpa armigera (Hubner), under various ratios of light to dark period of the LED pulse, 20ms:0ms (continuous lighting), 20ms:40ms, 20ms:80ms, 20ms:160ms and 20ms:400ms, using the actograph system in a 12-hour dark phase and compared the result with the 0ms:0ms (no lighting in the dark phase) using the same system. The results suggested that the flight activity of unmated males and females was generally at a lower level with 20:40 and 20:80 and it became clear to control the flight activity for five days continuously. However, the activity levels increased gradually unmated females with 20:0, 20:160 and 20:400, males with 20:0 and 20:400 in the dark phase with progress of a day, suggesting that the cotton bollworm became accustomed to the LED lighting conditions over days. Thus we considered that the flight behaviors (including feeding and reproduction behavior) of the adult moth was strongly suppressed under the conditions of 20ms:40ms and 20ms:80ms pulsed-LED light.

Keywords: *Yellow LED pulsed-lighting,Helicoverpa armigera,flight activity,actograph system,IPM*

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P4

Interactions between Tannic acid and *Bacillus thuringiensis* subsp. kurstaki KB100 against *Spodoptera exigua*

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Bacillus thuringiensis subsp. kurstaki KB100 isolated from the domestic soil have the most effective activity against larva of Spodoptera exigua. The tannic acid as protease inhibitor might be increased the efficacy of sublethal concentrations of B. thuringiensis. We identified tannic acid as protease inhibitor that increased the efficacy of sublethal concentrations of B. thuringiensis. Mixture of B. thuringiensis and tannic acid was investigated the mortality of S. exigua larva in the laboratory and field. When B. thuringiensis treated to 2nd larva of S. exigua, mortality was shown 54.4%. However, mixtures of B. thuringiensis with 4 and 40 mM tannic acid were increased mortalities to 2nd larva of S. exigua as 64.0 and 95.5%, respectively. In the welsh onion field, the accumulated mortalities of 3 times replicated with mixture of B. thuringiensis and 40 mM tannic acid were 83.9, 89.4 and 66.8% compare with 61.8, 80.4 and 47.3% as only B. thuringiensis treatment, respectively. Excessive degradation of activated toxin was observed in S. exigua midgut juice detected by SDS-PAGE. But tannic acid successfully prevented the excessive degradation of protoxin in S. exigua midgut juice by SDS-PAGE.

Keywords:*Bacillus thuringiensis, Protease inhibitor, Tannic acid, Spodoptera exigua*

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Seasonal Occurrence of *Riptortus pedestris* on Hairy Vetch and Its Development and Longevity on Host

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The seasonal occurrence of R. pedestris was investigated using pheromone traps baited with its aggregation pheromone in the fields with various crop compositions in 2010 and 2011. The incoming of R. pedestris into the experimental fields began to catch from late-April. After seed gathering season in mid-July, the incoming rate of R. pedestis decreased, and the activity was continually observed till early-December. Otherwise, during the two years of the investigation, thirty-eight species of hemiptera including R. pedestris collected, which indicated abundant species diversity in hairy vetch fields. However, in the laboratory, R. pedestris could not developed successfully to adult stage as a host plant with only seed and plant of hairy vetch, with decreasing survival rate after 4th instar and the failure of emergence to adult stage. Also R. pedestris showed higher preference on soybean than hairy vetch. Consequently, hairy vetch may be not a suitable host for the development and survival of R. pedestris. It is considered that R. pedestris that R. pedestris is a temporary visitor at the season of seed formation in hairy vetch fields.

Keywords:*Riptortus pedestris, occurrence, development, longevity*

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P4

Antifeedant activity and electrophysiological response of *Ailanthus altissima* against *Plutella xylostella* (Lepidoptera: Plutellidae)

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Antifeedant activity of crude extracts of Ailanthus altissima leaf was studied against third instar larvae of diamondback moth, Plutella xylostella L. (Lepidoptera: Plutellidae). During preliminary screening, the methanol extracts were tested at 5,000 ppm concentration. The methanol extract was further separated with hexane, chloroform, ethyl acetate, and water, in order. The chloroform solubles, which observed significant antifeedant activity, were subjected to open column chromatography on silica gel. And then five fractions (C1 ? C5) were obtained. Of these fractions, second fraction (C2) showed good antifeedant activity with 98.7%. The C22 and C23 fractions isolated via recycling open column chromatography on silica gel exhibited high antifeedant activity. These two fractions were separated using ODS open column chromatography. As a result, C231 fraction showed the highest antifeedant activity and also showed significant difference in medial styloconic sensilla of last larvae of P. xylostella at electrophysiological response using oscilloscope. The C231 fraction was re-isolated and purified by HPLC and recycling, finally obtained the bioactive compounds. The structure of bioactive compound isolated was confirmed by LC-mass, 1H-NMR (500 MHz), and 13C-NMR (100 MHz).

Keywords:*Plutella xylostella, Ailantus altissima, Antifeedant, Styloconic sensilla*

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P4

Comparison of Plasmid DNA and cry Gene in *Bacillus thuringiensis* subsp. aizawai KB098 and Their Mutants

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Bacillus thuringiensis subsp. aizawai KB098 is a high insecticidal activity against Spodoptera exigua. Insecticidal effective crystal was manifested (revealed) by cry gene. Cry gene is generally existed on the plasmid DNA of Bacillus thuringiensis. To find a factor which controls the formation of crystal, mutants were made by incubating KB098 at 42℃ (MKB-130) or irradiating UV (KBM-1). According to bioassay results, mutants didn't have toxicity and make a crystal. By extracting the plasmid DNA of KB098 and mutants, plasmid DNA encoded cry gene were confirmed. KB098 isolates had 4 cry genes, cry1Aa, cry1Ab, cry1C, cry1D, and also had been found seven plasmid DNAs. Contrary to plasmid DNA pattern of KB098, MKB-130 incubated at 42℃, however, left out the biggest plasmid DNA expected to encode cry gene. In result of PCR, 4 cry genes were not confirmed. KBM-1 irradiated UV didn't delete the biggest plasmid DNA expected to encode cry gene. As a result of plasmid DNA of KBM-1, there were conformed 4 cry genes similar to KB098. KBM-1 does not make crystal and there is no any toxicity against Spodoptera exigua. It is supposed that the sequence of plasmid DNA may be changed in the amplified cry gene.

Keywords:*Bacillus thuringiensis, Spodoptera exigua, mutant, cry gene*

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P4

Insecticidal Activity and Molecular Genetics Studies of *Bacillus thuringiensis* CAB530

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Bacillus thuringiensis is a gram-positive, aerobic and spore forming bacterium. B. thuringiensis is usually found and isolated from natural environments, for example, soil, warehouse, leaf surface, grasses and insect habitats, and world widely used as biotic pesticide. In this study, biological activities of B. thuringiensis CAB530 strain from Anomala albopilosa (Coleoptera) were examined. The number of colonies of B. thuringiensis CAB530 was 6.61 × 108 cfu/㎖. Bipyramidal crystal was produced from B. thuringiensis CAB530 by confirmed with phase contrast microscope. B. thuringiensis CAB530 observed the band at 130kDa carried out SDS-PAGE that is similar to B. thuringiensis subsp. Kurstaki that is an insecticidal activity against Spodoptera exigua. Five genes, for example, cry1Aa, cry1Ab, cry1C, cry1D, cry1F, cry1I were observed with PCR. LC50 values of B. thuringiensis CAB530 against S. exigua and Plutella xylostella showed a high toxical activity as 1.6 × 105 and 7.75 × 104 cfu/㎖, respectively.

Keywords:*Bacillus thuringiensis CAB530, Plutella xylostella, Spodoptera exigua, SDS-PAGE*

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P4

Effect of Pests Occurrence on the Surface Management Methods of Orchards

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Most of organic farming use the green manure crop for replace chemical fertilizer. We surveyed the species of disease and insect pest on green manure crop, pear and persimmon, and biological diversity as different surface management methods(hairy vetch planting, grass planting, clean-tilled crops) in orchard. The species of disease and pest on hairyvetch and grass was Bacterial spot, sclerotial rot, alfalfa weevil, Magoura crassicauda, and so on. These pests did not injury the pear and persimmon tree. The occurrence amount of major pests on pear and persimmon tree did not affected by different surface management methods. But, biological diversity in soil enhanced significantly at hairyvetch culturing orchard. In pear orchard, diversity index at hairy vetch planting, grass planting and clean-tilled crops was 1.66, 1.16, 0.92, respectively.

Keywords:*Green manure crop, Pesimmon, Pear, Biological diversity*

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P4

Insecticidal activity of plant extracts by farmers' made to manage brown plant hopper, *Nilaparvata lugens*

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The insecticidal activity of plant extracts by farmers' made on brown plant hopper(BPH), Nilaparvata lugens was evaluated in seedling bioassays. Mortality of single plant extract was very low but Japanese cypress and bamboo shoot extracts was high as 68% against BPH. The mixed extracts both Zanthoxylum piperitum and Ginko leaf extracts from brown rice vinegar was high mortality as 73%. Fermented pokeweed containing insecticidal soap was excellent mortality as 80% against BPH. Plant extracts from 95% alcohol had 4-fold higher mortality than that of water and vinegar. It suggested that plant extracts of Acorus gramineus, Melia Azedarach, Zanthoxylum piperitum, Chamaecyparis obtusa were high insecticidal activity against BPH.

Keywords:*insecticidal activity, brown plant hopper, plant extracts by farmers' made*

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Influence of aggregation pheromone trap on the distribution of egg parasitoids of *Riptortus pedestris* (Hemiptera: Alydidae) in soybean

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Aggregation pheromone of Riptortus pedestris (Fabricius) (Hemiptera: Alydidae) attracts its egg parasitoids, Ooencyrtus nezarae Ishii (Hymenoptera: Encyrtidae) and Gryon japonicum (Ashmead) (Hymenoptera: Scelionidae). Distribution and composition of these egg parasitoids were investigated in a soybean field where aggregation pheromone baited traps were installed for two consecutive years (2010 and 2011). Three traps without/with aggregation pheromone were installed on the edge of soybean field in before/after design. Parasitism changes were assessed on non-viable host eggs released at distances of 9, 18, 27, and 36 m from the trap. Higher egg parasitism (1.5 to 2 times) and adult emergence (1.8 to 2.6 times) was found up to 18 m distance from trap installation point in the presence of pheromone. Relative parasitism of the two parasitoids was different by years, but was not affected by the pheromone installation.

Keywords:*Ooencyrtus nezarae, Gryon japonicum, host egg release, parasitism, conservational biological control*

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P4

The Effect of Light-emitting diodes Source and Quality on Occurrence of Aphid

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Light-emitting diodes are used as the principal artificial light source in plant factory. The light environment created by a combination of artificial lighting influences directly or indirectly performance of pests. Aphids are major pests in many crop and fruit species, because they remove plant assimilates induce galls and transmit plant viruses. Thus, we investigated the effect of different LED light source and quality on aphid behavior in the LED chamber system. The nine light treatments were combined in three colors LED, duty ratio and irradiation time ratio. T1, T2 and T3 were treated by red+blue LED light and each duty ratio was controlled by 100%, 99% and 97% respectively. T4, T5 and T6 were adjusted for irradiation time ratio of the red and blue LED per minute and were 2:1, 5:1 and 10:1 respectively. T7, T8 and T9 were treated by red+blue+white LED light and each duty ratio was controlled by 100%, 99% and 97% respectively. As a result, the number of occurrence of the aphids in mixed LED light treatments was much more than blue LED alone, and especially, red+blue mixed light treatment had more aphids than red+blue+white mixed light gradient. Also, the number of aphids increased with higher red:blue ratio and duty ratio.The nine light treatments were combined in three colors LED, duty ratio and irradiation time ratio.

Keywords:*Aphid, LEDs, wavelength, duty ratio, light intensity*

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P4

Impact of resistance inducers for the management of late blight and sucking insects on Potato

Muhammad Usman Ghazanfar[1], Waqas Wakil[2], Yong Jung Kwon[3]

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Muhammad Usman Ghazanfar1, Waqas Wakil2, Yong Jung Kwon3 1 Department of Plant Pathology, University College of Agriculture, University of Sargodha, Pakistan 2 Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan 3 School of Applied Biology and Chemistry, Kyungpook National University, Daegu, Korea Abstract Pre-treatment of plants with chemical elicitors induces systemic resistance against plant pathogens and insect herbivores. The present study investigated the induction of defense responses and protective effects against Phytophthora infestans (Mont.) de Bary and sucking insects by the application of Salicylic acid (SA) and ?-aminobutrytic acid (BABA) under the green house conditions. Different dose rates of SA and BABA (2, 4 and 5 mM) were tested as foliage sprays. The application of both resistance inducers significantly suppressed the disease development 54.13% in case of SA and 67.47% in case of BABA by 15 days after inoculation with pathogen. The number and size of the lesions per plants were also greatly reduced by the application of treatments. Plants treated with BABA showed increased resistance against herbivory by aphids, thrips and whitefly with reduced fecundity of nymph, larvae and adults compared to SA treated plants. Changes in the activities of peroxidase and polyphenoloxidase were also studied. Potato plants treated with SA and BABA showed significantly higher peroxidase and polyphenoloxidase than water treated plants. From the present findings we speculate that metabolites produced as results of treatment with elicitors are responsible for protection against late blight and sucking insect pests and they can be used for successful disease/insect pest

Keywords:*Elicitors, SAR, peroxidase, polyphenoloxidase protection*

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P4

Studies on standard setting and mass rearing of natural enemies; *Aphidius colemani* Viereck, *Nesidiocoris tenuis* (Reuter) and *Chrysoperla canea* (Stephens)

Eunhye Ham[1], Cheol Hak Kim[2], Young Cheol Choe[3], Jun Seok Lee[4]

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Worldwide, more than 125 species of natural enemies are commercially available for biological control. In Korea, commercial use of biological control has seen a very fast development during the past 15 years. Currently, about 10 companies produce more than 35 species of natural enemies. The success of biological control depends upon the standard setting and effective mass rearing of natural enemies. In this work, we have evaluated the effects of a standard setting and mass rearing of natural enemies (Aphidius colemani Viereck, Nesidiocoris tenuis (Reuter) and Chrysoperla canea (Stephens)), applied with different techniques. The mass rearing process should be done with the acceptable quality. The quality control programmers should be designed in order to obtain acceptable quality, not necessarily the possible quality.As a result of intensive cooperation between researchers and the biological control practitioners we will develop simple and reliable quality control programs, standard setting and mass rearing for natural enemies. It is expected that these developments will result in a rapid improvement of the biological control industry.

Keywords:*Natural Enemy, Biological control, Rearing standard, Standard setting, Quality control*

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P4

Superior defense systems in maize landraces: a potential pest management tool for smallholder maize farmers in Africa

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Maize (corn), Zea mays, is a major staple and cash crop for millions of smallholder farmers in Africa. However, its production is severely constrained by lepidopteran stemborers, including Chilo partellus (Swinhoe). Control of these pests is difficult due to the burrowing into the plant stem by the larval stages of the pest. Feeding on the maize by stemborer larvae leads to an emission of herbivore induced plant volatiles (HIPVs) that attract the pest's natural enemies. Here we report discovery of maize landraces that react to egg deposition by C. partellus by increasing emission of HIPVs that attract parasitic wasps against both egg and larval stages of the pest. There is also reduced attractiveness of the plants for subsequent oviposition by the moths. This trait however seems absent in elite commercial maize hybrids. This therefore implies a sophisticated defence strategy whereby parasitoids are recruited in anticipation of egg hatching. These findings suggest that indirect plant defence traits may have become lost during crop breeding and could be valuable in new resistance breeding for sustainable agriculture.

Keywords:*maize, stemborer, HIPVs, control, Africa*

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P4

Study on resistance monitoring of major pests in rice to insecticides

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Rice stem borer (Chilo suppressalis), rice leaf folder (Cnaphalocrocis medinalis), and brown planthopper (Nilaparvata lugens) are three major pests on rice which heavily damage rice production annually in China. The resistance of three pests against insecticides were detected by topical application, leaf-dipping technique and rice stem-dipping method, respectively from 2006 to 2010 in Hubei province. The results showed that the Jianli population of rice stem borer had moderate levels of resistance to triazophos from 2007 to 2009. The rice leaf folder in this four field populations remained susceptible to abamectin with the LC50 values 0.26-1.24 mg/L, but the sensitivity of Zhijiang and Ezhou population to chlorpyrifos reduced year after year, indicating the rice leaf folder produced a certain resistance to chlorpyrifos from 2008 to 2010. The resistance of brown planthopper produced high level of resistance to imidacloprid in Hubei province from 2006 to 2010, some reached an extremely high level of resistance (389.19-fold). The resistance level and the resistance development speed of brown planthopper to buprofezin were discrepant among seven populations from 2007 to 2010, and some reached a high level of resistance. Gongan population developed high level of resistance to buprofezin (52.1-fold).

Keywords:*Resistance monitoring, Rice stem borer, Rice leaf folder, Brown planthopper, Field population*

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Developmental Control of brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae) by ingestion of dsRNA of arginine kinase gene

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RNA interference (RNAi) is a technological breakthrough that has been developed as a powerful tool for studying gene function and for the highly targeted control of insect pests. The effect of using a feeding-based RNAi technique to silence the Arginine kinase (AK) gene in Nilaparvata lugens was examined. Arginine kinase participates in cellular energy metabolism in invertebrates, which is analogous to the creatine kinase reaction. As s member of the phosphagen kinase family, it buffers cellular ATP levels by catalyzing the reversible phosphoryl transfer between ATP and arginine, leading to the production of phosphoarginine and Mg2+ADP. The completely coding region of Nilaparvata lugens ak is 1071 bp and encodes a protein of 357 amino acids. According the part of ak gene sequence which contain the active motif CPTNLGT, the gene specific primers were designed to amplify the sense strand and anti-sense strand DNA of ak gene. Because the designed primers include T7 promotor, the sense strand and anti-sense strand DNA of ak gene could be transcribed to dsRNA in vitro. Then, the purified dsRNA of ak gene was ingested into Nilaparvata lugens by artificial diet. The preliminary results show that sustained feeding the artificial diet contained ak dsRNA could killed 70% Nilaparvata lugens after 7 days. At the same time, the plant expression vector pds1301-ak that can express ak dsRNA was constructed and transformed the pds1031-ak vector into Agrobacterium tumefaciens.

Keywords:*Nilaparvata lugens, arginine kinase, RNA interference, plant expression vector, Agrobacterium tumefaciens*

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Resistance monitoring of *Plutella xylostella* to insecticides in central China

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Diamondback moth (DBM), Plutella xylostella L., is the most destructive pest on brassicaceae throughout the world. Because of the overuse of traditional insecticides, DBM's resistance to insecticides has attracted the world's attention. In this study, leaf-dipping method was used to monitor resistance of four field populations of DBM in central China (Wuxue, Yichang, Luoyang and Yueyang) to 10 kinds of insecticides (diafenthiuron, spinosad, chlorfenapyr, chlorfluazuron, cartap, Bt, indoxacarb, beta-cypermethrin, abamectin and tebufenozide) from 2008 to 2010. The results showed that resistances of four field populations of DBM in central China to beta-cypermethrin and Bt were in high to extremely high level, with resistant ratio ranged from 67.70- to 257.31-fold, 113.88- to 713.17-fold, respectively. Four field populations had moderate to high level resistance to chlorfluazuron, with resistant ratio ranged from 16.79- to 133.36-fold. Resistant ratios of four field populations to abamectin and tebufenozide ranged from 15.29- to 47.00-fold, 15.84- to 45.92-fold, respectively, while resistant ratios of field populations from Yueyang to cartap and indoxacarb, ranged from 11.30- to 26.45-fold, 10.13- to 12.88-fold, respectively, all indicated that resistances were in moderate level. Results also showed that field populations, collected from Luoyang, Wuxue and Yichang, were susceptible or had low level resistance to cartap, indoxacarb, diafenthiuron, spinosad, and chlorfenapyr. Field population of DBM from Yueyang had low level resistance to diafenthiuron, spinosad, and chlorfenapyr.

Keywords:*Plutella xylostella, insecticides, resistance, monitoring, leaf-dipping method*

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Comparative vertical and horizontal dispersion behavior of *Trichogramma brassicae* in the maize and rice fields

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Insect parasitoids have to disperse to locate host and host habitat. The parasitoid wasp Trichogramma brassicae is a dominant as well as the most important species of Trichogramma in Iran. For study the behavior of this parasitoid in the rice and maize fields, it is reared on the eggs of the grain moth. Wasps were marked using fluorescent powder and released from a central point. Parasitoids dispersal was studied using trap cards includes the eggs of the intermediate host at 2, 5, 9, 12, 18 and 25 m distanced from the central point and 30, 100 and 130 Cm above the ground on rice and 20, 50 and 100 Cm on maize fields. Trap cards were collected three days after releases and examined in the laboratory after six days. Analyses carried out using SAS software and GLM procedure. Results showed significant influence of heights, distances and the day after release as well as the crops. Mean comparison revealed different flight behavior of the parasitoids on different crops since the highest parasitism occurred in the medial heights in rice and in the first and third heights in maize. In addition, parasitism rates were not significant in 2-5 m and 2-9 m distanced from releasing point in rice and maize respectively, while the results at other distances were significant in both fields. Hence, vertical and horizontal dispersion of the parasitoid is influenced by the crop architecture, height and distance.

Keywords:*Dispersal, Trichogramma brassicae, maize, rice,*

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Sequential Sampling Plans for Estimating Population Density of *Heliothis viriplaca* Hufnagel (Lep : Noctuidae) on Rain-Fed chickpea

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The Pod borer, Heliothis viriplaca Huf, is considered one of the major insect pests of chickpea in Iran. Crop losses due to this pest can be substantial and may be prevented or reduced, by implementing an integrated pest management program (IPM). The success of IPM programs depends on good monitoring, so that sampling could be a key element. 28 chickpea fields (Kabuli Type) at the elevations of 1300 ? 1800 meter, were sampled during 2010 and 2011 in different localities of West Azarbaijan province of Iran. A 1 × 0.5 square meter sampling frame was used for sampling the H. viriplaca larvae. The maximum larval population (3.26 larvae/square meter) was recorded in Somaibradost district of Orumieh. However, the minimum larval population (0.14 larvae/square meter) was recorded in central district of Tekab. Taylor's power law regression model was used to describe the spatial distribution of larvae. B-value was 1.342 and indicated chickpea pod borer were aggregated in rain-fed chickpea. A fixed precision sequential sampling plan was developed using the parameters of Taylor's power low generated from the total number of larvae in the sampling frame. Stop lines were estimated at two precision levels (D) of 0.1 and 0.25. High-precision sampling plans generally necessitated impractical sample sizes, and thus we present a relatively low-precision level plan (SE/mean = 0.25).

Keywords:*Heliothis viriplaca, West Azarbaijan, Chickpea, Rain-Fed*

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Toxicity of chlorpyriphos and fenpropathrin to *Habrobracon hebetor* (Hymenoptera: Braconidae)

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[1]Chamran University, Iran, [2]Chamran University, Iran, [3]Chamran University, Iran

Habrobracon hebetor (Say) is a larval ectoparasitoid of several species of Lepidopteran pests including Tomato fruitworm (Helicoverpa armigera Hubner). The lethal effects of chlorpyriphos and fenpropathrin, which are overused in tomato production, were evaluated on the larval, pupal, and adult stages of the parasitoid H. hebetor in the laboratory. Dose-response bioassays have carried out on immature and adult stages by using dipping and contact residue methods, respectively. The results indicated that the slopes of dose-response line for all treatments were positive. This cleared that there was an increase in mortality regarding an increase in concentration for both insecticides. Comparative evaluation of the slope values of the three probit lines showed that the slope of the adult stage treated with chlorpyriphos and pupae treated with fenpropathrine are higher than the slope of the others. The slope value of the above treatments suggested that chlorpyriphos is more toxic to H. hebetor. The LC50 of chlopyriphos for three development stages (larvae, pupa, adult) was 0.18, 9.95, 2.91 mg AI L-1, respectively and the LC50 of fenpropathrine for three development stages was 1.80, 20.76, 12.44 mg AI L-1, respectively. The recommended concentrations for chlorpyriphos and fenpropathrine are 408 mg AI L-1 and 200 mg AI L-1, respectively. The LC50 of chlorpyriphos varied from 40 to 2000 times less than the recommended rate and 10 to 100 times less for fenpropathrine treatments on different life-history stages. So, the application of these insecticides is not recommended when this parasitoid is released against the pests.

Keywords:*Habrobracon hebetor, chlorpyriphos, fenpropathrin,Dose-response bioassays,*

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The Importance of the Parasitoid *Bracon hebetor* Say and the Pheromone Traps for Controlling *Ephestia* Spp in the Date Store

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Ministry of Science adn Technology, Iraq

The moth insects fig moth, Ephestia cautella, raisin moth, E. figulilella and carob moth, E. calidella, infest dates in date warehouses and orchards, this infestation was the main problem facing date trade in Iraq especilly after inhibiting using methyl bromide as an fumigant in the world, because it appeared to be Ozone depleting agentand carcinogenic agent. the results of using the parasitoidBracon hebetor and the pheromone traps in controllingmoth insects mentioned above were proved to be highly efficient in preserving date when they used together in the simulsted date warehouses in governorates:Baghdad, Karbala and Babylon for five months, the percentage of preserving dates were 91.6, 96.8 and 85.9% respectively, in comparison with 80.7 , 76.2 and 73.8% respectively, in the date warehouses in which the pheromone traps used only and with 70.2, 62.5 and 53.2%respectively , in the control date warehouses. These results suggest that the parasitoid B. hebetor and the pheromone traps should be recommended to be used for controlling moth insects mentioned above.

Keywords:*Ephestia spp, Bracon hebetor, Pheromone traps, Biological control*

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Parasitism of *Heliothis viriplaca* Hufnagel (Lepidoptera: Noctuidae) larvae by *Banchopsis crassicornis* Rudow (Hymenoptera: Ichneumonidae) in rain-fed chickpea

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Chickpea pod borer, Heliothis viriplaca Huf is a significant insect pest of chickpea in the south west Asia. A survey was carried out during 2008-2009 in the rain-fed chickpea fields of Western Azarbaijan province of Iran, to determine the parasitoids associated with the first or second instars larvae. First or second instars larvae were collected from the fields and fed on chickpea soaked seeds or on tender leaves inside glass tubes for emerging parasitoids. Results revealed that naturally parasitism of the larvae by Banchopsis crassicornis Rudow was considerable. Maximum parasitism (15.83%) was recorded in Somai Bradost district and the minimum (6.83%) was recorded in the Central district of Orumieh. Parasitized larvae continued feeding in the laboratory and developed to four or five instars inside the tubes. They stopped feeding a day prior to the emergence of the parasitoid from their bodies. In the laboratory conditions, none of the emerged larvae could enter the soil to form cocoons and all died. So that emerged larvae were warped in cotton and replaced in glass tubes. They readily formed brown spindle-shaped cocoons within 24 hours. The cocoons were kept for the emerging of adult insects. In field conditions, adult insects appeared in June, but in the laboratory conditions the adults, emergence took place in winter. The parasitoid had only one generation per year. B. crassicornis is distributed in Africa, Europe, Middle East and Caucasus and this is the first record from Iran. Also H. viriplaca is the new host recorded for this species.

Keywords:*Banchopsis crassicornis, Heliothis viriplaca, Iran, chickpea*

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IPM

P4

Study on synergistic effects of henna in enhancement of pathogenicity of *Bacillus thuringiensis* Berliner on third and fourth instars larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

Akbar Ghassemi-Kahrizeh[1], Nouraddin Shayesteh[2]

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In this Study the synergistic effects of henna powder in enhancement of pathogenicity of Bacillus thuringiensis var. tenebrionis serotype H8a, 8b and Bacillus thuringiensis var. kurstaki strain EG2424 on 3rd and 4th instars larvae of Colorado potato beetle, Leptinotarsa decemlineata (Say) (Col.: Chrysomelidae), was evaluated. Combination of 3 concentrations of henna powder (3000, 4000 and 5000 ppm) with bacteria (500 and 1000 ppm for 3rd and 4th instars larvae, respectively) was used against 3rd and 4th instars larvae in completely randomized plot design in laboratory conditions. The results revealed that there was a remarkable synergistic effect of henna powder on B.t. efficiency. The 3rd instars larval mortality for mixture of B.t. var. tenebrionis (500 ppm) with henna (5000 ppm) was 72.5% after 6 days, however the mortality of larvae treated with B.t. var. tenebrionis (500 ppm) alone was 17.5% in this time. The mixture of B.t. var. kurstaki (500 ppm) with henna (5000 ppm) had caused 85% mortality in the 3rd instars larvae during 6 days. This concentration of B.t. var. kurstaki produced 22.5% mortality on this larvae. The mortality for 4th instars larvae treated with B.t. var. tenebrionis (1000 ppm) was 12.5% after 6 days, however this rate for 4th instars larvae treated with mixture of B.t. var. tenebrionis (1000 ppm) and henna (5000 ppm) was 47.5% in this time. The mortality of 4th instars larvae treated with mixture of B.t. var. kurstaki (1000 ppm) with henna (5000 ppm) was 3.4 times as much of the mortality of fourth

Keywords:*Bacillus thuringiensis var. tenebrionis, Leptinotarsa decemlineata (Say), synergistic effect of henn*

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Study on morphological indices of Colorado potato beetle, Leptinotarsa decemlineata (Say) in west Azerbaijan province

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The Colorado potato beetle, Leptinotarsa decemlineata (Say), is a serious pest of potato, Solanum tuberosum L. worldwide. In this study, morphological indices of different biological stages of the Colorado potato beetle, Leptinotarsa decemlineata (Say) were evaluated in west Azerbaijan province. Weight, length and width of body for male and female adults, weight and length of body and head capsule diameter of different instars larvae, weight and length of pupae, egg length and number of egg in each egg mass were measured. The highest of weight, length and width average of body for adults were observed for female with mean values of 178.8±6.756 mg, 11.535±0.099 and 6.887±0.052 mm, respectively. The least and highest larval weight were observed for 1st and 4th instars with mean values of 1.300±0.082 and 88.667±6.191 mg, respectively. Average of head capsule diameter for 1st, 2nd and 3rd instars larvae were measured 0.684±0.011, 1.131±0.075 and 1.682±0.133 mm, respectively. Average number of egg in egg masses was measured 29.064±1.323.

Keywords:*Leptinotarsa decemlineata (Say), morphological indices*

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Control of eight species of important stored products pest insects with environmentally compatible compounds

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The toxicity of ozone and mixture of this gas with nitrogen, phosphine and carbon dioxide were evaluated under field conditions. In parallel experiments, the toxic impact of bran, sawdust, clay , flour and sayan® on eight important stored product insects Tribolium castaneum (Herbst), Sitophilus oryzae L., Oryzaephilus surinamensis (L.), Rhyzopertha dominica (F.), Lasioderma serricorne F., Cryptolestes ferrurgineus (Stephens), Callosobrochus maculatus (F.) and 3rd instar larvae of Plodia interpunctella (Hubner) was determined. The adults of T. castaneum, S. oryzae, O. surinamensis, R. dominica, L. serricorne, C. furrurgineus, C. maculatus and 3rd instar larvae of P. interpunctella were exposed either solely to nitrogen or as a mixture with phosphine and ozone, carbon dioxide. These experiments were carried out either in empty storehouse or in the storehouse contained foodstuffs, such date, wheat and rice, respectively. The toxicity of the gases was determined after 24 h of exposure. Cononcomitantly with these trials, the lethal impact of ozone on the S. oryzae and O. surinamensis in different depths of rice, wheat and date was estimated as well. The results of empty storehouse tests revealed that, the mixture of nitrogen with phosphine caused 84.73% mortality response on S. oryzae. In similar experiments in the presence of foodstuffs the highest mortality rates were occurred on S. oryzae and P. interpunctella 3rd instar larvae in the depths of 30, 35 and 40 cm in date.

Keywords:*Stored-product pests, gases mixture, ozone, phosphine, nitrogen, bran, Sayan*

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Resistance of different grape cultivars to grape erineum mite, *Colomerus vitis* (Acari: Eriophyoidae) in laboratory conditions

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The Grape Erineum Mite (GEM), Colomerus vitis Pagenstecher, is a serious pest of grape orchards (Vitis vinifera L.) in western Iran and sometimes causes a considerable damage to the crop. Initial observations in Hamedan showed the different levels of damage on different vine cultivars. Therefore the resistance of ten vine cultivars including White Thompson seedless, Red Thompson seedless, Fakhri, Yaghuti, Asgari, Muscat Gordo, Khalili, Flame seedless, Gazne and Shahani to GEM was examined to determine the potential use of resistant cultivars in pest management programs. The study was carried out under laboratory conditions at a temperature of 25±1°C, 65±5% R.H. and a photoperiod of L16: D8 h using pot disk method. Muscat Gordo, Gazne and White Thompson seedless cultivars with 100.4±0.45, 95.5±0.77 and 87.4±0.64 mite per leaf were shown the lowest and Shahani, Flame seedless and Yaghuti with 20±1.23, 24.2±1.24 and 24.7±2.15 mite per leaf showed the highest feeding repellency to the GEM, respectively. The highest oviposition rate was observed on M. Gordo and Gazne with 148.31±1.26 and 129.3±2.26 egg per leaf, respectively, and the lowest on Shahani with 10±1.37 egg per leaf. Our results showed that infested and non-infested leaf area of Shahani was not significantly different. Moreover, Shahani and Yaghuti cultivars had the highest bristle-like trichome density, whereas, M. Gordo and Gazne had the highest hair-like trichome density. According to the obtained results, Shahani and Yaghuti had the highest and M. Gordo and Gazne had the lowest resistance level to the GEM.

Keywords:*grape erineum mite, resistance, trichome, vine cultivars, western Iran*

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Total phenol and o-dihydroxy phenol profiles of black gram (*Vigna mungo* (L.) Hepper) genotypes infested by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleryrodidae)

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The principal limiting factor affecting production of black gram in northern India is whitefly, Bemisia tabaci (Gennadius) which causes direct injury by extracting sap from the plant tissue. The present study focused on deciphering comparative differences in total phenol and o-dihydroxy phenol (ODHP) profile of nine genotypes of black gram challenged by whitefly feeding. Total phenols and ODHPs were estimated from leaf samples collected at 30 and 50 days after sowing from whitefly infested and uninfested plants. Oviposition preference, nymphal and adult development of whitefly was also evaluated under screen house conditions. Correlation studies relating leaf content of black gram phenolics from different genotypes with whitefly population were also worked out. Biochemical analysis of black gram genotypes revealed that moderately resistant genotypes NDU 5-7 and KU 99-20 recorded higher per cent increase in their total phenol and o-dihydroxy phenol contents contents both at 30 and 50 DAS under whitefly stress conditions as compared to non-stressed plants. Total phenols and o-dihydroxy phenols showed significant negative correlation with whitefly population (nymphs and adults) suggesting that enhanced activities of these biochemicals may contribute to bioprotection of black gram plants against B. tabaci infestation. Hence, for developing tolerant varieties of black gram against whitefly, genotypes possessing higher total phenol contents can be selected for selection of desirable genotypes in whitefly resistance breeding programmes. Comparatively higher level of resistance in genotypes NDU 5-7 and KU 99-20 can serve as base for genetic improvement of black gram focusing on the development of resistant varieties to whitefly.

Keywords:*Bemisia tabaci, black gram, total phenols, ODHP, host-plant resistance*

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Insecticide resistance in Western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), on glasshouse cucumbers in Republic of Moldova.

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Introduction: Western flower thrips (WFT), Frankliniella occidentalis, is the dangerous pest of vegetable and ornamental crops in the glasshouses in many countries. In Moldova it episodically appears on vegetable crops in glasshouses. Mass reproduction of WFT was observed in 1996 on tomato [Verderevskaea et al., 1996] and in 2004 on cucumber [Moontean, 2004]. We studied the sensitivity of pest population to all four insecticides which are registered in our country for use in glasshouses: pyrimiphosmethyle (Aktellik, 50% EC), cypermethrin (Arrivo, 25% EC), imidacloprid (Confidor, 20% EC), and thiametoxam (Aktara, 25% WP). Methods: The study was carried out during 2004-2008 years. The sensitivity of thrips females to insecticides was estimated in the laboratory by the method of pest's settlement on insecticide-treated bean leaf discs. The median lethal concentrations of insecticide were determined by probit analysis and the rates of resistance were calculated. Results: WFT population collected on cucumbers was resistant to pyrimiphosmethyle (83x), cypermethrin (1700x) and imidacloprid (3x). The comparison of recommended and sublethal concentrations (LC95) of these insecticides has shown that they are not effective against F. occidentalis. The most toxic for thrips is thiametoxam. Conclusions: Studied WFT population is high resistant to organophosphate and pyrethroid insecticides. The resistance to imidacloprid develops in this population too. This leads to the need to expand the list of insecticides used against thrips in a covered ground. The number of insecticide treatments can be reduced by means of alternative methods of thrips control as the application of blue sticky traps with semiochemicals.

Keywords:*resistance, Frankliniella occidentalis, insecticides, glasshouse, cucumbers*

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Effect of sublethal doses of spiromesifen, spirotetramat and spirodiclofen on fecundity and fertility of female adults of *Tetranychus urticae* Koch (Acari: Tetranychidae)

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[1]*University of Tabriz, Iran,* [2]*University of Tabriz, Iran,* [3]*University of Tabriz, Iran*

Two spotted spider mite is an important pest of many agricultural and ornamental plants worldwide. Spirotetramat, spiromesifen and spirodiclofen are new pesticides with novel modes of action and low environmental risks. Sublethal effects of these compounds were assessed on two-spotted spider mite in laboratory. Up to 24h old adult females were placed on leaf discs treated with sublethal concentrations (LC10 or LC25). LC10 of spirodiclofen, spiromesifen and spirotetramat were 0.05, 0.5 and 7.7 mg ai/l, respectively. LC25 of these compounds were 0.36, 3.3 and 19 mg ai/l, respectively. Twenty four hours after exposure to treated discs, 20 females showing no visible symptoms of poisoning were transferred to untreated leaf discs. The leaf disks were changed daily for up to five days. The number of eggs laid during this period was recorded. At the end of 24 h exposure period, the survival rate was 0.96 in controls; and 0.83, 0.93 and 0.83 in females treated with LC10; and 0.76, 0.83 and 0.76 in females treated with LC25 of spirodiclofen, spiromesifen and spirotetramat, respectively. The survival rate was decreased in all treatments at end of five days. Total number of eggs laid per female decreased in all treatments compared with controls. The average number of eggs/ female/ day was 2.6 in controls; and 1.22, 1.80, and 1.64, in females treated with LC10; and 1.61, 1.87 and 1.49 in the females treated with LC25 of spirodiclofen, spiromesifen and spirotetramat, respectively. Egg hatching rate was slightly lower in treated females compared with controls.

Keywords:*Tetranychus urticae, spiromesifen, spirotetramat, spirodiclofen, fertility, fecundity*

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Lethal temperature for Japanese termite, *Reticulitermes speratus*, in infested wood using microwave energy

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Thermal death kinetics was examined to develop a heat treatment method to control the Japanese termite, Reticulitermes speratus, in wood packing materials for export goods. To determine the feasibility of microwave irradiation as an alternative treatment, we performed laboratory experiments on the blocks (200x200x250mm) of Canadian hemlock artificially infested with live termite workers with subsequent 2.45GHz microwave energy irradiation. The 100% mortality of treated termites was achieved above 55℃ of the measured wood temperature, regardless of irradiated time. All measurement points in the blocks reached 55℃ within 67-718 seconds with microwave irradiation by wood weight. We need further experimental verification, but commercial microwave (2.45GHz) treatment is a feasible alternative to conventional heat treatment to control Japanese termite on the wood packing materials for export goods.

Keywords:*Lethal temperature, Japanese termite, Microwave irradiation*

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Lethal temperature for Japanese pine sawyer, *Monochamus alternatus*, in infested wood using microwave energy

Sang Jae Suh[1], Jinseo Kim[2], Yong Jung Kwon[3]

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Thermal death kinetics was examined to develop a heat treatment method to control the Japanese pine sawyer, Monochamus alternatus, in wood packing materials for export goods. To determine the feasibility of microwave irradiation as an alternative treatment, we performed laboratory experiments on the blocks (200x200x250mm) of Canadian hemlock artificially infested with live larvae and pupae with subsequent 2.45GHz microwave energy irradiation. The 100% mortality of treated termites was achieved above 61℃ on larvae and 64 ℃ on pupae of the measured wood temperature, regardless of irradiated time. All measurement points in the blocks reached 64℃ within 77-815 seconds with microwave irradiation by wood weight. We need further experimental verification, but commercial microwave (2.45GHz) treatment is a feasible alternative to conventional heat treatment to control Japanese pine sawyer on the wood packing materials for export goods.

Keywords:*Lethal temperature, Japanese pine sawyer, microwave irradiation*

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Model development by control technique of natural enemy on the insect pest in cucumber greenhouses in Korea

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Aphis gossypii, Aulacorthum solani, Tyrophagus neiswanderi, Frankliniella occidentalis, and Palpita indica are the important pest species on cucumber greenhouse culture in Korea. When tested with natural enemies, they were successfully controlled in cucumber greenhouses forcing and semi-forcing cultured between December and March. For A. gossypii control, banker plants were planted at a rate of 2 pot per 330 m2. Aphidius colemani and Propylea japonica were released at a rate of 0.8 mumy per m2 and 0.2 larva per m2, respectively. For A. solani control, P. japonica was used at a rate of 0.2 larva per m2. Hypoaspis aculeifer and Amblyseius cucumeris were combined at a rate of 15.2 and 38.0 nymphs and adults per m2, respectively, for T. neiswanderi control while three natural enemies including H. aculeifer, A. cucumeris, and A. swirskii were combined at a rate of 15.2, 38.0, and 18.9 nymphs and adults per m2, respectively, for F. occidentalis control. Entomopathogenic nematodes (Steinernema carpocapsae GSN1 strain) were sprayed 2-4 times at a rate of 30,000 Ijs per m2 for the control of P. indica larvae.

Keywords:*Cucumber, insect pest, natural enenmy, model development, control technique*

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Occurrence of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Sindh, Pakistan

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university of sindh, Pakistan

Helicoverpa armigera (American bollworm) is major lepidopteron pest of cotton and other crops such as tobacco, chilies, cucurbits, pea, maize etc. Present study for occurrence of Helicoverpa armigera carried out in four cotton growing localities of Sindh, Pakistan (Saeedabad, Hala, TandoAllahyar and Jamshoro), during 2008, 2009 and 2010. We have observed population of Helicoverpa armigera by Sex pheromones traps for moth collection and by field observation for damage %. During present study it is observed that Helicoverpa armigera found only in Hala locality and it was not found in other three localities. Highest moth population and infestation recoded in month of August September 2008, 2009 and 2010. Presence of Alternative host plants (Pea, maize, and Cucurbits) and thick vegetation throughout year are the main cause for occurrence of Helicoverpa armigera in Hala locality.

Keywords:*Helicoverpa armigera, Lepidoptera, Pheromones, Infestation, Sindh, Pakistan*

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Management of mango hoppers *Idioscopus* spp. and *Amritodus* spp. (Cicadellidae: Hemiptera)

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Mango is seriously affected by several species of Cicadellids. The important species are Idioscopus nitidulus Lethierry, I. clypealis Lethierry, I. nagpurensis Pruthi, Amritodus atkinsoni Lethierry, and A. brevistylus Viraktamath. Hoppers on mango are widespread in Asia. The loss is due to the sap-feeding by adults and nymphs on flowers and shoots, which results in poor fruit-set and photosynthesis inhibition due to sooty mold on leaves. This yield loss ranges from 30 to 87%. Inadequate control leads to sooty mold staining the fruits reducing the commercial value. Studies at the Indian Institute of Horticultural Research, Bangalore, Karnataka, India showed that imidacloprid 200SL @ 0.25ml/litre of water as spray at early panicle emergence gave 100% control. Azadirachtin 0.03%@ 2ml/litre of water, neem soap @10gm/litre of water, suspension of Beauveria bassiana (MycoJaal)@5ml/litre of water gave effective control when populations are <4/panicle or when organic management is warranted, or in case of recurrence of hoppers after the first intervention. These have been found to be safe to pollinators also during full bloom when pollinators are active spray of any kind is not recommended. Further it was found that yellow sticky trap was useful in predicting the population on the tree for management decisions based on the model y = 1.3427x2 - 3.8731x + 1.3031; R2=0.86. It was found that under organic orcharding the predator Isyndus heros Fabricius survived to keep a check on the hoppers. The above management has been validated from 2009-2012. This study was supported by ORP-Sucking Insects and NABARD.

Keywords:*Mango, Hoppers, Bio-pesticides*

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A new technique to control and rejuvenate wood bored mango trees by *Batocera rufomaculata* (Fale) (Cerambycidae: Coleoptera)

Thippenahalli Narayanappa Shivananda[1], Abraham Verghese[2], Manjunath R Hegde[3], Amrik Singh Sidhu[4]

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India ranks first in terms of area and production of mango (Mangifera indica) in the world. Mango stem borer (Batocera rufomaculata) is considered a major pest resulting in partial drying or death of the plant due to damage of vascular bundles and/or secondary infection of Ceratocystis sp. To combat this problem, we have developed a product namely sealer cum healer comprising of few medicinal herbs namely Aloe vera, Withania somnifera to help the regeneration of damaged tissues and essential nutrients to rejuvenate and overcome deficiency of nutrients. The affected portion of the tree is cleaned by chipping the bark and the larva is removed/killed. After cleaning, the sealer cum healer is mixed in water and filled to the tunnels/holes by bringing to dough consistency. The tree trunk up to three feet is swabbed with the slurry of our product to absorb through stem/bark for correction of nutritional deficiency. The results from more than 2,000 trees in south India treated with our product have suggested that the borer problem and gummosis were controlled and also nutritional deficiency corrected in a year after treatment. In conclusion, we report that the sealer cum healer can effectively control stem borer and gummosis resulting in vigor by overcoming nutritional deficiency. The loss across India due to this borer is about 18 to 25%. Normally economically bearing older trees are affected. A single tree saved is equivalent to a saving of US $ 5000. The cost benefit amounts to 1: >100/tree/year. Support by NHM/NABARD is acknowledged.

Keywords:*mango, stem borer, gummosis, deficiency, healer cum sealer, India*

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Popularizing IPM of oriental fruit fly (*Bactrocera dorsalis*) in mango through an area wide approach in India

Thippenahalli Narayanappa Shivananda[1], Abraham Verghese[2], Manjunath R Hegde[3], Amrik Singh Sidhu[4]

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Oriental fruit fly is a major pest in several pockets of mango growing areas in India. IIHR being a premier Institute in the country has worked out an integrated pest management (IPM) schedule for its management. By following IPM the damage due to fruit fly was brought down to zero to five percent based on the trials conducted at multi locations in the country. The IPM consists of surveillance traps @ 8/acre, raking of soil below the tree, sanitation by destroying fallen fruits and application of bait splash (jaggery at 10% mixed with a mild toxicant) on the basal stem portions of tree at weekly intervals, when the fruits attain 60% maturity. This IPM was demonstrated through front line demonstrations (FLD) with the participation of farmers in nearly 57,000 acres, in area wide mode with the financial assistance of NABARD, Department of Biotechnology, National Horticulture Mission and ICAR, Govt. of India . The technology has been popularized in six districts of Karnataka, two districts of Andhra Pradesh and one district of Kerala in south India for the last three years. By implementing IPM, the damage has been controlled from 95 to 100%. Encouraged by the results, Governments of Odisha and Jharkhand have also implemented the IPM schemes in collaboration with IIHR. This IPM would lead to mitigation or fruit fly free regions in India besides enhancing yield by 30%. The cost benefit ratio was 1:.50. The trap technology was standardized with the collaboration of Imperial College London under DFID funding.

Keywords:*IPM, mango, oriental fruit fly, popularization, fruit fly traps, bait splash, sanitation*

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Evaluation of entomopathogenic fungi for the control of green peach aphid, *Myzus persicae*

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The green peach aphid, Myzus persicae Sulzer, is an economically important pest for greenhouse crops, because they cause direct damage by feeding on plant nutrients and indirect damage as transmits many virus vectors. Moreover, the overuse of insecticide has resulting in resistance among green peach aphid population and environmental pollution. Thus, the development of entomopathogenic fungi has received increasing interest as part of integrated pest management strategies as aphid biocontrol agents. In this study, we report the screening result of pathogenic fungi for the control of green peach aphid. Initial screenings were performed using 347 isolates of putative pathogenic fungi from Korea soils. As results, 20 isolates of entomopathogenic fungi were isolated from cadavers of green peach aphid supporting fungal conidiation. These isolates were identified as three strains of Lecanicillium attenuatum, nine strains of Beauveria bassiana, one strain of Metarhizium anisopliae, one strain of Metarhizium flavoviride, five strains of Paecilomyces lilacinus, one strain of Aspergillus sp. by microscopic examination, genetic sequencing of the ITS region and Universally Primed PCR (UP-PCR). Based on the screening results, twenty isolates were tested for their pathogenicity against adult green peach aphid, cold activity, thermotolerance, UV-B irradiation on conidia. All fungal isolates were pathogenic to green peach aphid but mortality varied with isolates. These entomopathogenic fungi may be useful to develop eco-friendly insecticide to control green peach aphid.

Keywords:*entomopathogenic fungi, green peach aphid, biological control*

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P4

Preparation and characterization of pheromone dispenser using nano materials

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The pheromone dispensers for Riptortus clavatus (Thunberg), Leptocorisa chinensis (Dallas), and Plautiastali (Scott) were synthesized using nano materials such as various divalent cations and hexadecyltrimethylammonium cation-exchanged zeolites A, X, and Y, MCM-41, and ETS-10. Each of pheromone component, (E)-2-hexenyl-(E)-2-hexenoate, (E)-2-hexenyl-(Z)-2-hexenoate, Myristylisobutyrate for Riptortusclavatus, 2-(E)-octadenyl acetate and octanol for Leptocorisachinensis, and methyl(E,E,Z)-2,4,6-decatienoate for Plautiastali, investigated maximum sorption capacity into/onto diverse materials by gas chromatography method. In case of all, various cation exchanged zeolites X and Y showed outstanding sorption contents comparison with other materials. In this work, we observed that the presence and absence of sorption sites and pore dimensions in nanoporous materials have the greatest effect on the rate of sorption, decreasing with decreasing pore diameter, and sorption sites for chemical interaction.

Keywords:*Pheromone dispenser, Nano materials, Sorption, Zeolite*

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Effect of pyriproxyfen on some physiological aspects of the pistachio fruit hull borer, *Arimania comaroffi*, pupae

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Pistachio fruit hull borer, Arimania comaroffi (Lepidoptera: Pyralidae), is one of the minor pests of pistachio trees, overwintering as pupa in silken cocoon in soil beside pistachio trees. In this study, effect of pyriproxyfen (an insect growth regulator), was investigated by measuring glycogen, glucose, lipid and protein contents of pupa. The last larval instar was exposed to pyriproxifen; the effect was studied in one week old pupa. Low molecular weight carbohydrate (glucose) as well as glycogen and lipid contents were measured by methods described by Warburg and Yuval (1996). Protein content of whole body was measured by Lowery method (Lowery et al., 1951). Glycogen content of treated pupae (3.11 mg/g fresh body weight) was significantly lower than control (8.51 mg/g fresh body weight).but no significant difference was observed between protein and lipid contents of treated pupa and control ($P>0.05$). Glucose content in pupae treated with pyriproxyfen (1.50 mg/g fresh body weight) was significantly different from control (2.99 mg/g fresh body weight). This study revealed that pyriproxyfen is able to significantly decrease carbohydrate level of treated pupa in relation to control.

Keywords:*Physiology, Pyriproxyfen, Arimania comaroffi*

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Insecticidal effects of *Thuja occidentalis* (Cupressaceae) essential oil on *Tribolium confusum* Duval (Col.: Tenebrionidae)

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In order to find recyclable, environment friendly and easy accessible insecticides, the essential oils of Thuja occidentalis L. (Cupressaceae), was used against the adult stages of Tribolium confusum Duval (Col.: Tenebrionidae). Analysis of Thuja occidentalis L. essential oil used for insect fumigation by phase gas chromatography revealed the presence of 22 compounds including α-thujone (49.64%), fenchone (14.06%), and β-thujone (8.98%). The experiment was conducted in 6 replications using a completely randomized design of factorial experiment. The essential oil was prepared by water distillation method. Experiment was carried out at 30±2 °C and 60±5% R. H. under dark condition. Concentrations are included 0.00277, 0.00352, 0.00425, 0.005, 0.00574 µl/l air and a control (untreatment), after 24 h, respectively. At the concentration of 0.00648µl/l air essential oil of Tribolium confusum Duval, caused 100% mortalities of adults, respectively. A value of 50% lethal concentration on adults was 0.111601µl/l air for Tribolium confusum Duval, respectively.

Keywords:*Thuja occidentalis, Tribolium confusum, Essential oil, Fumigation, Mortality, Lc50*

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Effects of Lufox on *Lobesia botrana* (Lepidoptera: Tortricidae) egg and larval stages

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The effect of the chitin synthesis inhibitor Lufox was evaluated against different developmental stages of lobesia botrana Den. & Schiff. (Lepidoptera: Tortricidae). In the present study the effect of the Lufox on the egg and larval stages were examined. Eggs included one to four days and Larvae were examined, including age one to age five.High activity was observed against L. botrana eggs with greater effect on 1-d-old eggs than on the other age classes and on eggs treated by direct contact rather than those laid on a previously treated surface. Eggs laid by treated adults showed the same effects during development as eggs treated by contact or those laid on a trcated surface. Larvae that emerged that from treated eggs could not perforate grape berries. Administrated into the diet, Lufox had a larvicidal effect, resulting in similar LC50 values for different instars for eggs: 0.35 ppm for one day old eggs, 0.39 ppm for two day old eggs, 0.43 ppm for three day old eggs and 0.49 ppm for four day old eggs and resulting in similar LC50 values for different instars for larvae's: 0.34 ppm for first instars, 0.36 ppm for second instars, 0.38 ppm for third instars, 0.41 ppm for fourth instars and 0.43 ppm for fiiveth instars.

Keywords:*Lobesia botrana, Lufox, LC50, Larval stages, egg stages*

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Interaction of *Bacillus thuringiensis* Berliner and the parasitoid, *Diadegma insulare* (Cresson) (Hym., Ichneumonidae) and diamondback moth, *Plutella xylostella* (L.) (Lep., Plutellidae)

Morteza Ebrahimi[1], Ahad Sahragard[2], Reza Talaei-Hassanloui[3]

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The survival of adult of D. insulare and adult Plutella xylostella (L.) treated with Bt-honey solution and the effect of Bt on immature stages of Diadegma insulare (Cresson) within host larvae, P. xylostella (L.) were evaluated in laboratory conditions in a growth chamber at 25 ± 1C, 65±10%, relative humidity (RH) and 16 : 8 h (L : D) photoperiod. Diamondback moth and Diadegma insulare pupae were collected from cabbage fields in Karaj (Iran). Commercial formulation of Bt. (Biolep®, Bacillus thuringiensis var. kurstaki) was used in these experiments. The value of LC50 for third instar larvae of P. xylostella was 209.59 ppm. DBM's and Parasitoid's adult survival at field rate of Bt was not significantly different from that of control. Formation of parasitoid's pupae in Bt-parasitoid combined treatments was significantly lower than parasitoid lonely. The Bt-parasitoid combined treatment significantly increased mortality of larvae of parasitoid within DBM larvae treated with Bt. Mean percentage mortality of larvae of DBM in LC30 and LC50 concentrations of Bt. combined with parasitoid was approximately 100% that was significantly higher than control (using parasitoid lonely). Our results showed that B. thuringiensis don't kill adults of DBM and parasitoid but kills D. insulare larvae indirectly by killing susceptible hosts in which they are developing. Successful use of multiple biological control agents will depend on close monitoring of their compatibility in the field.

Keywords:*Plutella xylostella, Diadegma insulare, parasitoid, Bacillus thuringiensis*

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Abundance of the beneficial insect predator Laius venustus (Coleoptera: Malchiidae) in Sudan and risk of some commonly used insecticides on its population

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The Melyrid beetle, Laius venustus was reported as an important natural enemy of the cotton Jassid (Jacobiasca lybica) and the cotton Whitefly (Bemisia tabaci) in Sudan. The abundance of the beneficial insect predator Laius venustus early in the cropping season 2004 was observed and surveyed on a groundnut field at the Gezira Research Farm (Wad Medani, Sudan). The predator appeared in large numbers in Mid-July and declined afterwards. During the survey similar numbers of adults of the predator were caught throughout the groundnut field. A relatively larger numbers were caught during mid-day compared to early in the morning. Dose-mortality tests with the most commonly used insecticides in Sudan were carried out. The results indicated a relatively higher toxicity of dimethoate and cypermethrin to field collected adults. Endosulfan was relatively safe to the tested beetles. Log dose ? Probit lines and the equations for each insecticide at different periods were determined. A susceptibility ratio based on the field recommended dosage rate was calculated

Keywords:*Risk assessment, Melyridae, Insecticides, Sudan*

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The application of insecticide against wires (*Agriotes meticulous*) at planting potatoes

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The protection of the yield of potatoes from different soil pests is of great importance from the scientific practical point of view here are such pests as larvae of beetles click (wires) false wires different types cockchafers, varmints larvae, stalk-grawing trowels (winter and gamma trowels and others). The scientific hypothesis and practice show that any water-soluable preparations (concentrates of emulsions, suspensions, colloidal and true solutions and others) applied in struggle against mentioned above soil pests, are absolutely perspectiveless because they are mainly contactable and available preparation of systematic effect don't give expected result owing to the characteristics of the environment (soil) in which these pests dwell. That is why the creation and application of powdery insecticides against soil pests is very actual/ For this purpose we have created the powdery preparation "Erdust", which combines 2 active matters at the expense of additional effect - phosphorus-organical compound any synthetic piretroids. After all-round prohibition of dusts DDT a GHCD there appeared the vital need for poundery preparations and such kinds powders were obtained for the first time. The researches were conducted according to generally accepted methodics and under conditions of field tests. It is ascertained that 2-times application of powderly preparation "Erdust" in the dozes: at planting potatoes -20-30 kg/ha and interrow processing -20-30 kg/ha fully exterminate the larvae of clicking-beetles (wirers). Its quality and the outer appearance of tubers improves.

Keywords:*agriotes meticulous,potato, dusts DDT, GHCD,Erdust*

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The biological protection of tomatoes from broomrape with the help of *.Piytomyza orobanhia* Kalt

Rakhmat Rasulov[1], Toshniyoz Goziev[2]

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Weeds-parsites cause considerably greater harm to plants-hosts, than the mast harmful no parasitical weeds independently from the degree of choking the sowings. Among weeds-parasites a special place is occupied by broomrape Which causes great damage to such agricultural crops as: tomatoes, tobacco, cabbages, pumpkins, carrots, sunflowers, peanuts and others. There exist several ways of struggle with broomrape Chemical (herbicides), biological (Front sight-fitomiza) organizational- economic (sowing resistant sorts) weeding and others. Taking into consideration mentioned above in 2010-2011 we conducted some experiments with testing different dozes of soil (triflurex) and exterminating effect (hurricane forte, round-up) herbicides on the plantings of tomatoes according to generally-accepted methodics. Besides, there were conducted experiments with growing and application natural enemy of broomrape front sight-fitomiza by methodics B.G.Bronshteign on the plantings of tomatoes in the neigh bouring farm (spatial isolation between experiments comprised 5-6 km). The first raising hopes results were obtained from the 2 exeriments. So, during experiments with herbicides there were revealed optimum dozes (round-up an triflurex), which allow to exterminate broomrape without any damage for tomatoe-plants. The biological effectiveness comprised (in average yearly) on herbicides comprised, accordingly 65,3 % and 63,5. During the experiment with front sight-fitomiza there were achieved positive results (biological effectiveness comprised in average yearly 55,2m %). Unfortunately the combination of chemical and biological methods of struggle against broomrape In one experiment didn't give positive results, owing to the sensibility of front sight-fitomiza to herbicides. But the work on this trend will be continued.

Keywords:*tomatoes,fitomiza,agricultural crops,broomrape Chemical*

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Compatibility of *Lecanicillium longisporum* with acetamipride and imidaclopride under laboratory condition

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The entomopathogenic fungus Lecanicillium longisporum is one of the facultative insect pathogens with significant host range and host specifity. Conidia survival may be effected by environmental factors or by bio-pesticides and chemical products used to protect crop plants. Lack of attention to this point can be an obstacle to the development of fungi in the field to be considered. In this research compatibility of mentioned fungi with acetamipride and imidaclopride and effect of these insecticides on conidial germination and vegetative growth were studied. The formulations of insecticides were tested in tree concentration (mean concentration-MC, half MC and twice the MC). The study was conducted in Completely Randomized Design (CRD) with 4 replications. The results indicated that acetamipride and imidaclopride in half MC compatible with L. longisporum (isolate LRC190). However, based on the results imidacloprid compared acetamipride is adaptable with L. longisporum. This insecticides could be used simultaneously in low concentration with this entomopathogenic fungus in integrated pest management.

Keywords:*Lecanicillium longisporum, Entomopathogenic fungus, insecticides, Campatibility.*

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Bionomic of *Tetranychus urticae* and *Scolothrips longicornis* on bean and cucumber

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ABSTRACT: Bionomics of two-spotted spider mite, Tetranychus urticae Koch, and Scolothrips longicornis Priesner was studied in the southeast of the Tehran province, Varamin from 11th July to 22th September 2010 on bean and cucumber. The mean population densities of the overall life stages of T. urticae and S. longicornis per leaf on cucumber (42.10 and 2.87, respectively) were significantly more than bean (6.18 and 1.32, respectively). The spatial distribution of T. urticae and S. longicornis was determined by the following two methods: Taylor's power law and Iwao's patchiness regression. The spatial distribution pattern of T. urticae and S. longicornis using Taylor's power law and Iwao's patchiness on cucumber was aggregated and on bean was random. The linear regression between the predator and prey population densities indicated a density-dependant predation by S. longicornis on T. urticae. Spatial distribution parameters of T. urticae and its predator can be utilized to outline a sampling program, estimate population density of these mites and efficiency of the predator for using in IPM programs.

Keywords:*Tetranychus urticae, Scolothrips longicornis, population density, spatial distribution, density depe*

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Preliminary observation on the relation of BPH resistance and RRSV infection in rice entries carrying different BPH resistance genes

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4 BPH resistance genes (Bph-1, Bph-3, Bph-10 and Bph-15) were regarded as BPH-resistant donors for hybrid rice breeding to cross with elite hybrid maintain or restorer lines in March 2011 in Hainan Island and 43 hybrid combinations (F1) were obtained. After germination, sprout seeds were sown in plastic box. All 1160 seedlings at 3-leaf stage of the 43 hybrid F1 were inoculated with BPH nymphs in laboratory in June 2011 in Hangzhou with a standard seed-box screen evaluation method. The offsprings of BPH caught from field on October 2010 and reared in rice seedlings of TN1 were used for infestation. Fifteen to twenty nymphs in the age of the 2nd to 3rd instar per seedling were infested. Though, the 4 BPH resistant genes are dominant, higher mortality rates of F1 plants overwhole were reached, the average survival rate of all lines holding the 4 resistant genes was only 18.97% with a range of 8.23 - 30.59%. The F1 plants survived from BPH infestation were transplanted into the field at 6- to 7-leaf stage. A high incidence of RRSV symptoms appeared in these plants in 2 to 3 weeks after transplanting. Partial of the RRSV infected plants could not enter the stage of panicle initiation, while others could reach this stage but could not fill seeds, only a few seeds could be harvested from some main stems of few small plants). The average percentage of plants with normal panicle filled (percentage of RRSV disease resistant) F1 plants was only 25.91%.

Keywords:*Hybrid Bph RRSV resistant*

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Effects of two isolates of *Beauveria bassiana* (Balsamo) Vuillemin on *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) larval stage

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The primary damage of Leptinotarsa decemlineata (Say) (Col.: Chrysomelidae) is leaf feeding on potato. The fungus generally infects its host through the insect integument. Germ tubes from the conidia penetrate the cuticle of the insects surface body. Nowadays biocontrol by entomopathogenic fungi could be considered as a suitable alternative method to chemical insecticides or at least could be used as an alternative to the chemical insecticides. Beauveria bassiana is a fungus commonly found in soils worldwide. Insect's susceptibility varies in different strains of the fungus. In this study, two DEBI007 and IRAN1217C isolates of B. bassiana were used in pathogenicity trials on L. decemlineata second larval stage. Treatments were conducted at 25±1°C, 75 ± 5%RH and 16:8 light/dark. The mortality was counted daily. Revision surgery was 15 days and dead larvae were singled out for possible contamination incident within a week of wet filter paper were kept in Petri dishes. The contact and immersion method was applied against second instar larvae by suspension them in prepared pathogenic fungal. Application of spore suspension with the concentrations of 107, 3.1×107, 108, 3.1×108, 109 spores/ml of fungal isolates comprising two isolates of B. bassiana on Colorado beetle showed that LC50 belonging to IRAN1217C was 2.4 ×107 spores/ml, and in the case of DEBI007 the LC50 was 1.4×107 spores/ml. The results show that the fungi isolate DEBI007 has more insecticidal efficacy and effectively play its role in integrated pest control programs.

Keywords:*Biological control, Entomopathogenic, Beauveria bassiana, Leptinotarsa decemlineata*

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Investigation of lethal effect of Proteus and Movento on tobacco aphid (*Myzus nicotianae* Blackman) under greenhouse condition

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Tabacco is one of the important agriculture and economical plants of the world and the income from its different products forms an important part of the national revenue of the countries which produce it. Myzus nicotianae is one of the dominate and most damaging of tobacco pests that in addition to sucking the sap, has a role in transmitting major viruses. Farmers use Primicarb, Imidiaclopride and Confider pesticides to prevent aphid damage although some degrees of resistance have been seen towards these pesticides. In this study the effect of two new insecticides namely, Proteus and Movento were investigated in order to use more effective insecticide and decreasing the times of poison spraying for the purpose of making the product more economical and maintaining the health of the farmers and the environment. In order to identify the poison level (LC50) of Proteus and Movento, bio-assessing experiments with spraying method on the wingless female adult under labratory conditions was carried out 25±5°c, 65±5% R.H, and 16:8 photoperiod of L:D. The results were analized by Probit method and Spss software. The results indicate that LC50 in 48 hours for Proteus and Movento was respectively 0.00034 and 0.00032. As the results indicate, Movento was more toxic on adult stage of Myzus nicotianae than Proteus.

Keywords:*Myzus nicotianae, Primicarb, Imidiaclopride, Proteus*

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Cloning and expression of a novel cry1 I gene from *Bacillus thuringiensis* isolates and its toxicity against *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera : Curculionidae) and *Helicoverpa armigera*

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six isolates of Bacillus thuringiensis from Andaman and Nicobar Islands which were previously characterized by PCR analysis for the presence of Coleopteran active cry genes were used for Cry1I full length gene amplification. A 2.16-kb DNA fragment of Cry1I gene was PCR amplified, cloned in expression vector pQE 80 L, and then used for transformation of E. coli M15 cells. The optimum expression was obtained with 1 mM IPTG at 370C for 3 h. The sequence of the cloned crystal protein gene showed almost complete homology with a Cry1I toxin gene from Bacillus thuringiensis var. kurstaki, with scattered mutations in the toxic region. The deduced sequence of the protein has homologies of 91.0% with Cry1I and Cry1Ia, and 98.0% with Cry1Ib.Cloning of this gene may help to overcome the increasing resistance of pests to currently used insecticides. Based on the results obtained, the PCR method may be a valuable and reliable tool for specific detection and identification of cry1I genes. The toxicity of Bt recombinant protein was determined against first instar larvae of Myllocerus undecimpustulatus undatus Marshall (Coleoptera: Curculionidae) and Adults; Helicoverpa armigera Hubner (Noctuidae: Lepidoptera) at 310µg/mL and 15.5µg/mL respectively. The novel cry1I gene will be an important resource in constructing genetically engineered bacteria and transgenic plants for biocontrol of insect pests and Bt based biopesticidal formulations, aiming to reduce the use of chemical insecticides.

Keywords:*Bacillu thuringiensis, coleoptera, toxicity*

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Investigation of lethal effect of Proteus and Movento on tobacco aphid (*Myzus nicotianae* Blackman) under greenhouse condition

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Tabacco is one of the important agriculture and economical plants of the world and the income from its different products forms an important part of the national revenue of the countries which produce it. Myzus nicotianae is one of the dominate and most damaging of tobacco pests that in addition to sucking the sap, has a role in transmitting major viruses. Farmers use Primicarb, Imidiaclopride and Confider pesticides to prevent aphid damage although some degrees of resistance have been seen towards these pesticides. In this study the effect of two new insecticides namely, Proteus and Movento were investigated in order to use more effective insecticide and decreasing the times of poison spraying for the purpose of making the product more economical and maintaining the health of the farmers and the environment. In order to identify the poison level (LC50) of Proteus and Movento, bio-assessing experiments with spraying method on the wingless female adult under labratory conditions was carried out 25±5°c, 65±5% R.H, and 16:8 photoperiod of L:D. The results were analized by Probit method and Spss software. The results indicate that LC50 in 48 hours for Proteus and Movento was respectively 0.00034 and 0.00032. As the results indicate, Movento was more toxic on adult stage of Myzus nicotianae than Proteus.

Keywords:*Myzus nicotianae, Movento, Proteus*

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An investigation on aphids (Homoptera) biodiversity of apple trees in Urmia region

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Biodiversity inciudes the diversity of species and their abundance. Biodiversity of homopteran species of apple tree in Urmia region was determind by measuring the number and abundance of each species collected during 2010-2011. During 2010-2011, investigation, nine species belonging to two families were collected and identified as follows: Family: Aphididae Eriosoma lanigerum (Hausmann) Myzus persicae (Sulzer) Dysaphis plantaginea (Pass.) Dysaphis affinis (Mordv.) Dysaphis Devecta (Walker) Dysaphis brancoi (CB) Aphis fabae (Scop.) Aphis pomi (Dedeer) Family: Lachnidae Pterochloroides persicae (Cholodovsky)

Keywords:*Biodiversity, Homoptera, Aphididae, Apple trees,Urmia*

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Study on aphids (Homoptera) fauna of wheat form in West Azerbaijan, Iran

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During in 2010-2011, a faunstic study was carried out to collect and identify aphids on wheat forms in west Azerbaijan region. A total of sexth species belonging to onefamily were collected and identified as follows: Family: Aphididae Diuraphis noxia (Mordvilko) Sitobion avenae (Fabricius) Metopolophium dirhodum (Walker) Schizaphis graminum (Rondani) Rhopalosiphum padi L. Rhopalosiphum maidis Fitch

Keywords:*Aphid, Faunstic, Wheat, West Azerbaijan*

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Investigation of population fluctuations of sunn pest,s egg parasitoids (Hymenoptera; Scelionidae) and estimating parasitism rate in central regions of West Azerbaijan Province, Iran

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Sunn pest (EurygasterintegricepsPuton) is the most important insect pest of wheat in Iran. This pest has numerous natural enemies within which egg parasitoids are undoubtedly the most important of them. In the survey population fluctuations and estimating parasitism rate of these insects were investigated in three regions of West Azerbaijan Province, Iran (Uremia, Naghadeh and Mahabad) in 2011. The egg traps were used at weekly intervals in fields. The parasitoids emerged from the traps were identified. Overal five species including Trissolcusgrandis(Thomson), T. djadeshko(Ryachovski), T. rufiventris (Mayr), Ooencyrtusfecondus(Ferriere) and O. teleminocida(Vassiliev). T.grandiswas the most common species followed by T.djadeshkoin all regions. Estimating parasitism rate were investigated in three regions: Uremia 65%, Naghadeh 60% and Mahabad 58%.

Keywords:*Sunn pest, Natural enemies, Parasitoids, Estimating parasitoid*

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Survey of the caused damage by the birds on the sunflower fields in different planting dates in west Azarbaijan

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Birds are among the most important pests in the fields under sunflower cultivation that lead to huge annual losses of the crop. Out of the deleterious birds of sunflower fields, house sparrow (Passer domesticus) causes the most abundant damage in the regional fields of sunflower. The present investigation was carried out to evaluate house sparrow damage on sunflower cultures in the Agricultural and Natural Resource Research Station of Khoy since 2006 in a biannual research project. The main plots included four seeding dates with 15 day intervals and the accessory plots were three sunflower cultivars Alstar, Euroflor, and KSC43/128. The cultivars were seeded in the Khoy station and the rates of bird damages were calculated at the end of seed maturity period. Based on variance analyses of data, seeding dates and cultivars had highly significant impacts on sunflower yield and the damage rate. The highest protected yields were resulted from the first and the second seeding dates exceeding 2500 kg/ha. However, the most damage rates were also calculated with the first and the second seeding dates, respectively rated as 96% and 93%. The damage rate in the second year was higher than that in the first year. Among the tested cultivars, the hybrid Alstar was of the highest damage rate (92%), and two hybrids Euroflor and KSC43/128 were of the least damage rates.

Keywords:*Sunflower, Damage and Birds*

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The review of insecticidal activity and oviposition deterrence of essential oils from *Artemisia dracunculus* L and *Mentha piperita* L against *Callosobruchus maculatus* F.(Col.; Bruchidae)

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Using chemical materials for controlling pests has caused many side effects on organisms and environment. In this research, lethal and sublethal effects and oviposition deterrence activity of essential oils Artemisia dracunculus L. and Mentha piperita L. on the Cowpea weevil adult Callosobruchus maculatus were evaluated. Testing was performed at 26 ±1 0C, 70± 5 % RH with seven concentrations of each essential oil. Fumigant toxicity of essential oils was assessed on the adults of the pest. LC50 values for each essential oil were calculated at different time of exposure, LC50 values for A. dracunculus 24.54 µl/lair after 24h and for M. piperita were 21.21 µl/lair after 9h. Sublethal concentration (8.48 and 13.63 µl/lair respectively for essential oils of A. dracunculus and M. piperita) of essential oils negatively affected the longevity and fecundity of female adults. The sex ratio of C. maculatus offspring was not significantly affected by essential oils. Also use of the concentrations (0.02, 0.06 and 0.1 µl/g) showed great potential of these essential oils for oviposition deterrence on this pest as cause 100 and 94.5% oviposition deterrence in 0.1 µl/g For A. dracunculus and M. piperita, respectively. The results indicated high insecticide properties of both essential oils on Cowpea weevil so the use of essential oil can be recommended as a low-risk pesticide and stored pests control with high impact.

Keywords:*essential oils, toxicity, Artemisia, Mentha, Coleoptera*

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Insecticidal activity and GC/MS analysis of three essential oils on cowpea weevil *Callosobruchus maculatus* F. (Col.; Bruchidae)

Khadijeh izakmehri[1], Moosa Saber[2], Mohammad Bagher Hassanpouraghdam[3], Ali mehrvar[4]

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Cowpea weevil, Callosobruchus maculatus F. (Col.; Bruchidae) is one of the most important stored product pests that make damages on seeds. Due to side effects of synthetic insecticides, more attention is devoted to the use of essential oils. Fumigant effects of the essential oil of three plants including Artemisia dracunculus L., Heracleum persicum Desf and Eucalyptus sp. were assessed on adults C. maculatus. The essential oils were extracted by Clevenger type apparatus. Essential oils were analyzed by GC and GC/MS. There were 28 chemical compounds in A. dracunculus , 21 chemical compounds in H. persicum and 47 chemical compounds in Eucalyptus sp. that are mainly among monoterpenoid groups. Acute toxicity bioassay was carried out to calculating LC50. The LC50 values were 24.5, 136.36 and 26 and µl/lit air in 24 h for A. dracunculus, H. persicum and Eucalyptus sp., respectively. The LC50 value was compared in 3 different temperature and relative humidity. The temperature affected the toxicity of the essential oils significantly but humidity did not. LT50 values for A. dracunculus , H. persicum and Eucalyptus sp. were 8.9, 10.93 and 6.3 hours, respectively. The results showed that the lethal effects of the essential oils were increased by increasing temperature and humidity. Our results showed that the essentials were effective against the pest and reduced the pest infestation. Application of these oils would be useful in IPM programs.

Keywords:*Essential oil, toxicity, bruchidae, stored product*

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Lethal, sublethal and combination effects of azadirachtin and *Bacillus thuringiensis* Berliner on cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

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The Cotton bollworm, Helicoverpa armigera Hubner is a polyphagous and cosmopolitan pest that causes damages on various plants. The continuous use of insecticides has caused resistance and made environmental pollution. In this study, lethal, sublethal and combination effects of azadirachtin (BioNeem®) and Bacillus thuringiensis sub sp. kurstaki (Safer®) were evaluated on third larval instar of H. armigera. Bioassay tests carried out by stomach method and the artificial diet glomeration at 26±1oC, 70±5% RH and a photoperiod of 16:8(L:D)h. The LC50 value of azadirachtin and Bt were 12.95 and 96.76 µg a.i./ml, respectively. The interaction of LC20 values of the insecticides caused 56.7% mortality on third instar larvae. The LT50 value of azadirachtin and Bt were 4.78 and 3.59 day, respectively. The results showed that sublethal concentration (LC30 values) of azadirachtin and Bt negatively affected the larval and pupal weight and additively affected larval and pupal duration of H. armigera. The longevity and fecundity of female adults was significantly affected by the insecticides. The least of adult emergence ratio and pupation ratio was observed in azadirachtin. The results indicated that both of insecticides had high potential for controlling H. armigera.

Keywords:*cotton bollworm, IPM, biorational insecticides*

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Acute and population level toxicity of azadirachtin and cypermethrin on larval ectoparasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae)

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Habrobracon hebetor Say is an important ectoparasitoid of various lepidopteran larvae. Lethal and sublethal effects of cypermethrin and two different formulations of azadirachtin (BioNeem® and Neem Guard®) were evaluated on H. hebetor under laboratory conditions. The adults were exposed to fresh residues of the insecticides applied on glass plates at 26±1oC, 70±5% RH and photoperiod of 16:8 (L:D) h. Based on the dose-response study, the LC50 value of cypermethrin, Neem Guard and BioNeem were 5.42 , 43.54 and 10.16 µg a.i/ml, respectively. The effect of azadirachtin and cypermethrin on larval stage was tested by dipping the larvae to field recommended concentration (FRC). Emergence rate was reduced by 97.56, 39.02 and 36.58 % at cypermethrin, Neem Guard and BioNeem, respectively. For assessment of the sublethal effects, adult wasps were exposed to an LC30 of either insecticide for 24 h and then the demographic parameters of live wasps were studied. The results showed that cypermethrin and two formulations of azadirachtin negatively affected fecundity and fertility of females adults. And also the intrinsic rate of increase (rm), finite rate of increase (λ) and doubling time (DT) were significantly affected by the insecticides. The sex ratio of H. hebetor offspring was not significantly affected by the insecticides. The intrinsic rate of increase for the control, cypermethrin, Neem Guard and BioNeem exposed populations were 0.179, 0.16, 0.143 and 0.149 female offspring per female per day, respectively. The results indicated that these insecticides and H. hebetor could constitute compatible components for IPM.

Keywords:*parasitoid, toxicity, IPM, population parameters*

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Insecticidal effects of 1, 8-cineole essential oil on *Tribolium confusum* Duval (Col.: Tenebrionidae) under laboratory conditions

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The uncontrolled use of synthetic insecticides is a great hazard for the environment and consumers. Essential oils were introduced as low toxic agents against mammals and non-targeted insects. The scale and duration of this disruptive activity depends on the concentration of the active agent in air and on its stability. During recent years, some plants have been receiving global attention and their secondary metabolites have been formulated as botanical pesticides for plant protection since they do not leave residues toxic to the environment, have lower toxicity to mammals and medicinal properties for humans. In order to determine their repellency and stability as repellents against Tribolium confusum du Val. (Col.: Tenebrionidae) adults, the activity of 1, 8-cineole essential oils. In this research fumigant toxicity of essential oil 1, 8-cineole was studied against 1-7 days old adults of the confused flour beetle, Tribolium confusum du Val. Bioassays were carried out at 28±1 ºC and 60 ± 5% R. H. under dark condition adopting a complete randomized block design with five concentrations of essential oil were tested in four replications with 20 adult insect in each replication. Mortality of adults was recorded at 24 h. The LC50 and LC95 value was 59.27 and 160.6 µl/L air respectively. A direct relationship between mortality rate and dose was detected. The findings of current study suggest that essential oils could also be used as insecticide against the stored product pests.

Keywords:*Essential oil, 1, 8-cineole, Tribolium confusum, insecticidal activity*

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Preliminary studies on prevailing species of rose stem borer sawflies in Urmia, Iran

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In a Three-year investigation during 2007-2009, efficiency of some biological and ethological characteristics of Stem Borer Sawflies' prevailing species was studied in order to diminish pest population density in Urmia rose-gardens. Genus of this economically important wasp was recognized, Syrista sp., which belongs to Cephidae family. Species of Syrista sp. has sexual dimorphism and one generation in a year. Daily average advance of larva in feeding tunnel was estimated about 5 millimeters. Yearly average infestation of rose-bushes, adult sex ratio of male/female and spatial distribution pattern were %39.33, 1: 3.71 and clumped (Negative binominal), respectively. The mortality of overwintering larvae inside infested branches by observation of ordinary autumnal pruning was %20. Treatments of larvae spring control tests were included pruning of branches´ tips, spraying pit of branches by Azinophos methyl of Iran made, spraying by Azinophos methyl of German produce in addition to control treatment. This test was conducted by completely randomized design in soft and hard branches. According to results and counting of larval mortality after 96 hours and mortality percent of treatments were 99.9 and 99.9, 75 and 25, 85 and 30, 0 and 0, respectively. Therefore infested branches tip pruning technique had the best effect between treatments in both conditions on early June and can be recommended, to be valuable either as a component of any IPM program or an independent control tool and particularly is effective due to lowering risk of environmental contamination through reducing pesticide application.

Keywords:*Stem Borer Sawfly, Syrista sp., rose, control, biology, pruning*

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Life table parameters of *Agonosena bimaculata* on Khinjuk trees , *Pistacia khinjuk* and cultivated pistachio

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The Khinjuk psylla , Agonoscena bimaculata Mathur was reported as a pest of the Pistacia khinjuk . Life table laboratory condition (27.5 C° , 55±5 % RH and 16 L: 8 Dh) . At Pistacia vera the intrinsic rate of increase (rm) , finitrate of increase (λ) , net reproduction rate (R0) , mean generation time (T) and population doubling time (DT) were 0.12 , 1.12, 81.56 ,37.5 and 5.9 days , respectively , and at Pistacia khinjuk they were 0.11, 1.12 , 75.1 , 36.41 and 5.8 days , respectively . The resullts of this study could be seen as a starting point for further research in order to improve the management of A.bimaculata populations.

Keywords:*Agonosena bimaculata, life table parameters,Pistacia khinjuk*

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Field Evaluation of *Beauveria bassiana* and *Verticillium lecanii* against jasmin whitefly, *Aleuroclava jasmine* on citrus

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Ministry of Science & Technology, Iraq

Pathogenicities of entemopathogenic fungi, Beauveria bassiana and Verticillium lecanii were evaluated against jasmin whitefly, Aleuroclava jasmine infested citrus trees under field conditions of three different locations. Results indicated that parasitization percentages of B. bassiana on eggs and nymph were relatively comparable to that of V. lecanii with some differences between locations. The parasitization percentages of eggs were, in general significantly lower than that of nymphs. It was found that parasitization of both fungal species tested, increased significantly with time. The results showed the probable negative effect of high temperatures and low humidity on both fungal parasitization potentials.

Keywords:*Beauveria bassiana, Verticillium lecanii, Aleuroclava jasmine*

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Efficacy of *Bacillus thuringiensis* against the larvae of *Helicoverpa armigera* and its compatibility with *Metarhizium anisopliae* and chlorantraniliprole used against this insect pest

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This study was designed to evaluate Bacillus thuringiensis subsp. kurstaki and its combination with Metarhizium anisopliae and new chemistry insecticide - chlorantraniliprole against the larvae of six field population of tomato fruitworm Helicoverpa armigera Hubner (Lepidoptera: Noctuidae). The two dose rates of B. thuringiensis (0.5, 1 µg g-1), one of both M. anisopliae (1.3×106 conidia ml-1) and chlorantraniliprole (0.01 ppm) were applied alone and in combination with each other against 4th and 5th larval instars. The mortality was observed after every 24 h and the larvae were kept for pupation. The mortality was observed in Rawalpindi with lowest pupation rate by applying the combined concentrations of B. thuringiensis and chlorantraniliprole, while, the lowest mortality was observed in Gujranwala among all the tested populations. The results clearly showed that these agents may interact with other but more focused research is required to optimize the dose rates against the larval instars of tomato fruitworm.

Keywords:*Entomopathogenic fungi, Bacillus thuringiensis, new chemistry insecticide, tomato, fruitworm, field,*

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Comparative study on the effect of *Bacillus thuringiensis* var. tenebrionis, imidacloprid and phosalone on different larval instars of *Leptinotarsa decemlineata* (Say) under laboratory conditions

Akbar Ghassemi-kahrizeh[1], Shirin Mohammadzadeh[2], Mahmoud Pooryousef Myandoab[3], Shahram Aramideh[4]

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In this study the susceptibility of different larval instars of Colorado potato beetle, Leptinotarsa decemlineata (Say) to Bacillus thuringiensis var. tenebrionis Ber. (as a microbial insecticide), imidacloprid (as a new insecticide) and phozalone (a widely used insecticide against this pest) was investigated in laboratory conditions. For this reason the larvae were reared on potato leaves in laboratory conditions. Then different larval instars were separated by measuring the diameter of head capsules and were used in bioassay tests. The different concentrations of commercial formulation of B.t., biobit (B.t. var. tenebrionis), confidor 200 SC (imidacloprid) and zolone EC 35% (phosalone) were tested on 1st, 2nd, 3rd and 4th instars groups of larvae. The LC50 values for these larval instars for B.t. var. tenebrionis after 144 h were 252.07, 613.21, 1731 and 3006 ppm, respectively. This criterion for imidacloprid was estimated 17.58, 41.35, 56.76 and 81.27 ppm, respectively for different larval instars in the same order after 72 h. The LC50 values for these larval instars for phosalone after 72 h were estimated 447.46, 697.88, 1020 and 1297 ppm, respectively.

Keywords:*Colorado potato beetle, Bacillus thuringiensis var. tenebrionis, imidacloprid, phozalone*

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Study on morphological indices of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) in west Azerbaijan province

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The Colorado potato beetle, Leptinotarsa decemlineata (Say), is a serious pest of potato, Solanum tuberosum L. worldwide. In this study, morphological indices of different biological stages of the Colorado potato beetle, Leptinotarsa decemlineata (Say) were evaluated in west Azerbaijan province. Weight, length and width of body for male and female adults, weight and length of body and head capsule diameter of different instars larvae, weight and length of pupae, egg length and number of egg in each egg mass were measured. The highest of weight, length and width average of body for adults were observed for female with mean values of 178.8±6.756 mg, 11.535±0.099 and 6.887±0.052 mm, respectively. The least and highest larval weight were observed for 1st and 4th instars with mean values of 1.300±0.082 and 88.667±6.191 mg, respectively. Average of head capsule diameter for 1st, 2nd and 3rd instars larvae were measured 0.684±0.011, 1.131±0.075 and 1.682±0.133 mm, respectively. Average number of egg in egg masses was measured 29.064±1.323.

Keywords:*Leptinotarsa decemlineata (Say), morphological indices*

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Study on synergistic effects of henna in enhancement of pathogenicity of *Bacillus thuringiensis* Berliner on third and fourth instars larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

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The synergistic effects of henna powder in enhancement of pathogenicity of Bacillus thuringiensis var. tenebrionis, and Bacillus thuringiensis var. kurstaki on 3rd and 4th instars larvae of Colorado potato beetle, Leptinotarsa decemlineata (Say), was evaluated. Combination of 3 concentrations of henna powder (3000, 4000 and 5000 ppm) with bacteria (500 and 1000 ppm for 3rd and 4th instars larvae, respectively) was used against 3rd and 4th instars larvae in completely randomized plot design in laboratory conditions. The results revealed that there was a remarkable synergistic effect of henna powder on B.t. efficiency. The 3rd instars larval mortality for mixture of B.t. var. tenebrionis (500 ppm) with henna (5000 ppm) was 72.5% after 6 days, however the mortality of larvae treated with B.t. var. tenebrionis (500 ppm) alone was 17.5% in this time. The mixture of B.t. var. kurstaki (500 ppm) with henna (5000 ppm) had caused 85% mortality in the 3rd instars larvae during 6 days. This concentration of B.t. var. kurstaki produced 22.5% mortality on this larvae. The mortality for 4th instars larvae treated with B.t. var. tenebrionis (1000 ppm) was 12.5% after 6 days, however this rate for 4th instars larvae treated with mixture of B.t. var. tenebrionis (1000 ppm) and henna (5000 ppm) was 47.5% in this time. The mortality of 4th instars larvae treated with mixture of B.t. var. kurstaki (1000 ppm) with henna (5000 ppm) was 3.4 times as much of the mortality of fourth instars larvae treated with B.t. var. kurstaki (1000 ppm).

Keywords:*Bacillus thuringiensis var. tenebrionis, Leptinotarsa decemlineata (Say), synergistic effect of henn*

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IPM

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Genetic rice breeding for resistance to the *Oryzophagus oryzae* in Brazil

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The Brazilian rice water weevil (RWW) Oryzophagus oryzae (Col.: Curculionidae) is the major insect pest in flooded rice in South of Brazil. Adults feed on rice leaves. However, is the larval root pruning that promotes the main yield losses. Cultivars resistant to RWW is considered a promising alternative to reduce the insect damage to the flooded rice and consequently the insecticide use, operational costs and risks of environmental contamination. The rice resistance to the RWW belongs to the antixenosis, antibiosis and tolerance types. The antixenosis and antibiosis would reduce the insect oviposition level and cause the larval death in the growth initial phase, respectively. The tolerance is characterized by root system recovery after the larval pruning. Studies started in 1980 have indicated a possibility to obtain rice resistance to RWW based on antixenosis, antibiosis and tolerance mechanisms. Initially commercial varieties, advanced breeding lines, land races, alien resistance sources are evaluated in rice field conditions, under natural insect infestation. Selected genotypes of the preliminary evaluation are revalued under field and greenhouse conditions, in cages, with high insect infestation and with and without host plant choice. The cultivars Dawn and BRS Atalanta were selected as antixenosis and antibiosis resistant sources while BR-IRGA 413 as tolerant resistant source. These cultivars are being used as a source of genes in a population of recurrent selection in rice breeding program of Embrapa. In this context, the objective is to present the state of research on genetic resistance of rice to the RWW in Brazil.

Keywords:*Flooded rice, Insect, Root damage, Plant resistance, Antibiosis, Tolerance*

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A effective pesticide? Spirotetramat for the control of the *Cacopsylla chinensis* Yang & Li (Hemiptera: Psyllidae)

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The spirotetramat is a new two-way systemic and absorptive insecticide and miticide developed by Bayer company, belong to Tetronic acid. Here, the field control efficacy of the spirotetramat to the Cacopsylla chinensis was studied. The result show that spirotetramat has a good control efficacy long after the pray. The control effects of 2000, 3000 and 4000 concentrations are above 89% after 28 d, and among 81-89% after 91 d, obviously higher than the control pesticides of fenpropathrin, deltamethrin, imidacloprid and emamectin-benzoate. Our research indicate that the spirotetramat could successfully control the Cacopsylla chinensis in the whole year with one time spray.

Keywords:*two-way systemic insecticide, spirotetramat, Cacopsylla chinensis, control efficacy*

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Mechanism of resistance in wild relatives of pigeonpea against *Maruca vitrata* Geyer (Lepidoptera:Pyralidae)

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Maruca vitrata Geyer (Lepidoptera: Pyralidae) is one of the most destructive insect pest of pigeonpea owing to the feeding habit on the reproductive structures of the crop. The levels of resistance are considerably low in the cultivated germplasm and development of resistant varieties with incorporation resistant genes from wild relatives can be an important component in insect pest management. Hence, the ovipositional and feeding preference of adults and larvae on the pigeonpea wild relatives viz., Cajanus scarabaeoides and Rhyncosia rufescens were evaluated in comparison with the cultivated variety VBN (Rg) 3 under field and laboratory conditions. Under field conditions, there was no oviposition on C.scarabaeoides and R. rufescens consequently no damage under field conditions as against 12 egg/10 cm twig/plant and 20 floral webs/plant with one larvae/web in VBN (Rg)3. Under laboratory conditions, both under no choice and free choice test, there was no oviposition on the wild relatives as against 8eggs/twig in VBN (Rg) 3. The feeding damage of the third instar larvae on the flowers were highest on VBN (Rg) 3 (80.00%), followed by R.rufscens (40.00%) and C.scarabaeoides (37.78%) while on the pods the larvae failed to feed on the wild relatives as against 47 per cent damage on VBN (Rg) 3. The results clearly indicate the ovipositional and feeding non preference in the wild relatives.

Keywords:*pigeonpea, wild realtives, maruca vitrata, resistance*

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Seasonal migratory behaviors of *Mythimna separata* (Lepidoptera:Noctuidae) in Northeast China

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Seasonal migratory behaviors of Mythimna separata (Walker) in Northeast China were studied by long-term observation using a vertical-looking radar (VLR) and light trapping in Jilin province, in combination with analysis of large-scale wind systems and trajectory simulation based on GIS. Results showed that migration of M. separata only occurred on a few nights during each of three distinct migration periods, that it flew at obviously different altitudes depending on season and time of night, and that its migratory behavior was significantly influenced by meteorological conditions, especially the large-scale atmospheric circulation. The long-distance migrations were wind-assisted, and most of the individuals were able to continue in flight throughout the night. The migration altitude in spring was mainly 300-600 m, while in autumn it was relatively low, mainly below 500 m. Radar echoes in summer showed obvious stratification, indicating that the migrants were sometimes concentrated into two or more layers with a maximum height of up to 1000 m. Trajectory analysis showed that in spring, populations of M. separata flying over the radar station originated from the southwest, probably from Shandong Province in mid-July, however, populations were locally produced and dispersed relatively short distances under the influence of convective weather. In autumn, populations of M. separata originating from the Hulun Buir region of Inner Mongolia were observed to fly on northwesterly winds to the southeast of Jilin province.

Keywords:*Mythimna separata, migration, radar observation, trajectory analysis*

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Insecticidal potentiality of *Datura innoxia* leaf extracts against the adult cluster bug *Agonoscelis pubescens* (Thunberg) (Heteroptera: Pentatomidae)

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The cluster bug (Agonoscelis pubescens) is a major pest of Sorghum bicolor and some other crops in Sudan. Despite the high cost of its chemical control, the population of the pest that attacks the crop retains more or less similar trend every year. Such demerits of synthetic insecticides have advocated the search for effective ecologically sound alternatives. Therefore, laboratory experiments were carried out to evaluate the insecticidal potentiality of Datura innoxia leaf extracts against the adult A. pubescens, as compared with a standard insecticide. The leaves were extracted with water and two organic solvents (hexane and ethanol). The parameters included; comparisons of three application techniques (spraying, dipping and filter paper methods) and bioassays of the different extracts. In both cases, mortality means were recorded at different intervals (2, 3, 5 and 7days) from treatments. The results revealed that the spraying of A. pubescens bugs on plant leaves gave the best effect than the other tested techniques. Regarding bioassays, leaf hexane extract exerted significantly the highest mortality levels as compared with the other extracts, though was surpassed by the insecticide. In all extracts, the mortality rates generally increases with increasing concentration and exposure time. Moreover, Datura extracts also seem to manifest a hormonal effect on the insect as deformed hind legs were appeared in some treatments. In conclusion, the study proved that leaves of D. innoxia contain variable biological activities on the pest, and invites more research to isolate and characterize their active compounds for proper utilization as natural biocides.

Keywords:*Agonoscelis pubescens, Datura innoxia, bioassays, mortality effect, hormonal effect.*

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Evaluation of synthetic (Indoxacarb, Flubendamide, Spinetoram, Flafenoxuaron, Chlorfenypyr, Leufenuron and Coragen) and Microbial pesticides (Emamactin and Spinosad) against *Spodoptera litura*

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Experiments were conducted to evaluate the effect of synthetic and microbial insecticides against the larval stage of Spodoptera litura. The experiments were performed in the Plant Protection Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. Uniform population of laboratory culture (F3 Generation) of S. litura was used. Twenty 3rd instar larvae were released in each treatment. Filter paper method was used. Different concentrations of synthetic insecticides, Indoxacarb (1.25, 5.25. 10 and 20ppm), Flubendamide (3.15,6.25,12.5, 25 and 50 ppm), Spinetoram (12, 24, 48,96 and 192 ppm), Flafenoxuaron (6.25, 12.5, 25, 50, 100 ppm), Chlorfenypyr (20.6. 41.5, 82.5, 165 and 330 ppm) Leufenuron (6.25, 12.5, 25 and 50ppm), Coragen (10,20,40,80, 160ppm) and that of the microbial insecticides; Emamectin (2.375, 4.75, 9.5, 19.5 and 38 ppm) and Spinosad (12, 24, 48, 96 and 192), were used to determine the larval mortality. It was found that at lowest concentrations, Leufenuron proved to more effective causing 33% of larval mortality while at highest concentrations of Indoxacarb (20 ppm) and Chlorfenpyr (100 ppm), 100% mortality was observed. The result showed that synthetic insecticides have high acute action as compared to the microbial insecticides.

Keywords:*Microbial pesticides, Synthetic Insecticides, Larval mortality*

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The synergitic effect of Henna powder on increasing efficacy of *Bacillus thuringiensis* var. Kurstaki against third larval instar of Pistachio white leaf moth *Ocneria terebinthina* (Lep.: Lymanteridae)

Zahra Sheibani Tezerji

Islamic azad university, Rafsanjan branch, Iran

In this research the susceptibility of the third larval instar of Ocneria terebinthina (Lep.: Lymanteridae) to Bacillus thuringiensis var. kurstaki alone and in the mixing with henna powder (Lawsonia alba (Litraceae)) was investigated. The eggs of the insect were collected from orchards where located around Anar in Rafsanjan. The third larval instar was separated by measuring the head capsule width, and then used in all bioassays. In bioassay, LC50 value of Bt in third larval instar was determined by using treated pistachio offshoot. The data was analyzed by log-probit transformation using the MSTATC software. LC50 value for third larval instar was estimated 2817.34 ppm. Then the synergitic effect of henna powder on efficacy of Bt was evaluated. Several doses of henna powder were combinated to the minimum lethal dose of Bt for third larval instar (more resistant than first and second larval instars) in complete randomized block design in three replicates. The results revealed henna powder has synergitic effect on Bt. So that, mixture of Bt (1000 ppm) and henna powder (6000 ppm) caused 81.11% mortality whereas Bt only caused 17.77 mortality. According to the collected data in this research and the selective nature of Bt it can be concluded that this microbial compound in combination with henna powder synergist can control of Ocneria terebinthina reasonably.

Keywords:*Bacillus thuringiensis var. kurstaki, Ocneria terebinthina, LC50, Henna powder, Synergist*

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Reproductive toxicity of imidacloprid and bifenthrin to the biological control agent, *Chrysoperla carnea*

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Lethal effect of two insecticides; Imidacloprid and Bifenthrin, was checked on survival and reproduction of Chrysoperla carnea adults. C.carnea adults were collected from field and reared in the IPM Station, BZU, Multan. Experiments were completed on F1 generation of the test organism. The adults were provided fresh water and artificial diet. Bifenthrin 10EC and Imidacloprid 20SL were used for experiment. Four replicates of each concentration with three pairs of adults were used. Different concentrations of Imidacloprid (500, 250, 166.6, 125 and 100ppm) and Bifenthrin (500, 250, 125, 83.3, 62.5 and 50ppm) were applied so as to evaluate the possible effect of both of these insecticides on the biology of C. carnea. Treated water was fed to the adults for four consecutive days. After feeding, the biology of insect was observed. The results showed that in the case of Imidacloprid, mortality was at its maximum on the field dose, i.e. 500 ppm, whereas the hatching percentage was also significantly reduced at the same dose rate. In the case of Bifenthrin, highest mortality was recorded at 500 ppm and there is a significant decrease in egg laying and percentage hatching. Imidacloprid proved to be more toxic as compared to the other insecticide tested. Hence, it can be concluded that the synthetic insecticides has a definite impact on the biology of biological control agents, as in the case of C.carnea.

Keywords:*Reproductive toxicity, Bio-control agent, Toxicological effects, C.carnea, Imidacloprid, bifenthrin*

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Analysis of physio-morphic traits in different host plants associated with resistance to *Phenacoccus solenopsis*: An invasive pest in Pakistan

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Phenacoccus solenopsis Tinsley(Pseudococcidae:Hemiptera) is sucking insect pest and polyphagous in nature. Twenty five different host plants were screened against P. solenopsis and their physicomorphic traits were determined to correlate their role against pest population. Among tested plant species, most favorable host plants of mealy bug on the basis of population were Gossypium hirsutum (69.74±10.5), Solamum melongena (62.41±10.0), Helianthus annuus (61.83±10.0), Hibiscus rosa-sinensis) (61.1±10.0) and Lantana camara (55.83±9.75) but the least preferred plants were tandla Digera arvensis (1.57±1.00) and Conyza bonariensis (3.83±1.75). Significant variations were observed in all the morphological plant characters recorded from different plant species. The maximum leaf thickness per plant was 2.04±0.23µm in plant species S. melongena while minimum thickness of leaf was recorded from lehli (0.25±0.02µm). Maximum trichome density per plant was 444±72.4 of sunflower followed by 411.6±19.6, 399±52, 391.6±22.0, in C. bonariensis, Abelmoschus esculentus and Withania somnifera respectively but minimum 2.33±1.45 of Chinopodium morale, followed by 2.66±1.4, 3±2.08 of , Portulaca oleracea and Trianthema portulacastrum) respectively. The maximum hair length per plant 2.62±0.07 mm was of sunflower followed by 2.55±0.03 mm in Okra, 2.53±0.06 mm in both S. melongena and G. hirsutum. It was concluded that plant characters including trichome density and hair length favor mealy bug population showing r-values of 0.357*, 0.190 respectively, but leaf size, leaf width (leaf area) and leaf thickness exerted negative effect on pest population showing r-values of -0.172, -0.285 respectively.

Keywords:*Phenacoccus solenopsis, invasive species, physicomorphic traits, resistance*

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Investigation on the most appropriate pattern, sample unit and sample universe to estimate *Tetranychus urticae* Koch. population in common bean, *Phaseolus vulgaris* fields.

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Abstract: Two spotted spider mite, Tetranychus urticae Koch. is one of the most important pests of common bean in most areas of Iran, including the Lorestan province. The most appropriate pattern, unit and universe sampling of mite population were investigated in common bean fields in Borujerd, Dorud and Azna in 2008. In this study, two patterns of movement in the field (X shape and zigzag) were used. Statistical analysis showed that, there was no significant difference between these two patterns, in related to CV, RV and RNP parameters. The results showed that, sampling should be done from top, middle and bottom of plant height. In addition, comparison of three sample units, 1.5 leaves per plants (a half leaf from each plant height), 3 leaves per plants (a leaf from each plant height) and 6 leaves per plants (two leaves from each plant height) showed that there is no significant difference between CV and RV amounts of each female, nymph and egg populations with related to CV, RV and RNP parameters. But sample unit 1.5 leaves with the highest amount of RNP, showed significant difference comparison to two sample units 3 and 6 leaves. Based on this research, sample unit with 1.5 leaves (three half leaves, left and right consecutively from three plant height) is recommended in sampling of T.urticae populations in common bean fields.

Keywords:*Tetranychus urticae, Sample unit, Sample universe, Common bean*

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Pathology

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ABCC2 functions as a receptor for *Bacillus thuringiensis* Cry toxin.

Shiho Tanaka[1], Kazuhisa Miyamoto[2], Hiroaki Noda[3], Ryoichi Sato[4]

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Transgenic Crops which produces Bacillus thuringiensis Cry toxins (Bt-Crop) has been used widely because of its advantages as a safe and environmentally friendly product. However, with the expansion of planting the Bt-Crop, evolution of Bt-Crop resistant strains in the field population has become clear. Recently, ABC transporter subfamily C2 (ABCC2) which had never been thought as a molecule associated with the mode of action of Cry toxins was reported as a causal factor of Cry1A toxin's resistant insects. Here, we report evidences that Bombyx mori ABCC2 (BmABCC2) functions as the most important receptor in this insect for Cry toxins and mutations occurred in the toxin-binding regions and ATP binding cassette in this molecule could cause generation of resistant strains. When BmABCC2 alone or both BmABCC2 and cadherin-like receptor, BtR175 was ectopically expressed in Spodptera frugiperda Sf9 cells, the cells became susceptible to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa and even Cry8Ca, but not to Cry3Bb. On the contrary, BtR175 could not offer susceptibility to the cells. From these, susceptibility of cells which expressed both BmABCC2 and BtR175 seemed correlated well to susceptibility of B. mori larvae. Alanine-replacement mutations made in the extra-cellular loop 3 and 5 regions of BmABCC2 removed the susceptibility-giving function from BmABCC2, suggesting that these are toxin binding regions. Mutations introduced in Walker A and Signature motif region which are critical to ATPase activity of ABCC2 also removed the susceptibility-giving function from BmABCC2. Now, we are trying to clarify the real meaning of these phenomena.

Keywords:*Bacillus thuringiensis, Cry toxin, ABC transporter, Bombyx mori*

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Pathology

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Characterization of cell line developed from the neonate larvae of *Papilio demoleus* Linnaeus (Lepidoptera: Papilionidae)

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A new cell lines RIRI-PaDe-3 developed from the neonate larvae of Papilio demoleus Linnaeus was established in the modified Grace medium supplemented with 20% fetal bovine serum (FBS) and incubated at 28 ºC. The cell line consisted of short spindle and round cells, both of them were wall-adhering cells. The chromosome numbers of cells varied widely from 28 to 136. The population-doubling time (PDT) of cells was 47.6 h at 71th passage. Comparison of nuclear ribosomal ITS sequences found in the cell line with those found in neonate larvae confirmed that the cell line was of P. demoleus Linnaeus origin. This cell line was susceptible to the Apocheima cinerarius nucleopolyhedrovirus (AciNPV) and Autographa californica nucleopolyhedrovirus (AcNPV). We conclude that the RIRI-PaDe-3 cell line will be a useful tool for studying virus replication.

Keywords:*Papilio demoleus Linnaeus, Neonate Larvae, Cell Line, Characterization*

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Pathology

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Economized media for spore production of white mascardine, *Beauveria bassiana* (Balsmo) Vuillemin and its pathogenicity on *Brontispa longissima*

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Mass-production of entomopathogenic microorganisms is a prerequisite for their use in microbial control of pest insect programs. Four different types of media, corn, sorghum, the Sao Hai rice variety and the broken unpolished rice pieces, were laboratory evaluated for economized spore production of the entomopathogenic white mascardine, Beauveria bassina in four incubation periods, 7, 14, 21 and 28 days. There was a positive trend of spore produced, 2.14 x 109, 3.06 x 109, 3.86 x 109 and 4.13 x 109 spores/ml when cultured on broken unpolished rice pieces medium, increased significantly over other tedt media. As a result, the broken unpolished rice pieces medium was the most suitable one for the spore production among the test media. Subsequent test of pathogenicity of the fungi (density 1x 108 spores/ml) with different ages of spores, 7, 14, 21 and 28 days old, on adults of Brontispa longissima Gestro (Coleoptera: Hispidae), an economic insect pest of coconut, exhibited a quadratic equation, % mortality of B longissima = 7.9429+8.7592(day)-0.2332(day)2, [R2 = 0.8134 (P<0.01)]. It caused 90% mortality of the adult of B longissima when its spores 18.78 days old under control environment (25+2 0C, 75+5%RH, 24 hr light).

Keywords:*media, Beauveria bassiana, pathogenicity, Brontispa longissima*

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Pathology

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Analysis of two molecules determining susceptibility to Cry1 toxin in *Ostrinia scapulalis*

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Cry toxins are crystalline insecticidal proteins produced by Bacillus thuringiensis. Each of them has unique specificity of target insects. This specificity is largely determined by the specific interaction between Cry toxins and their receptors located at the surface of midgut epithelial cells. The lepidopteran insect, Ostrinia scapulalis, is natural resistant to Cry1Aa. We hypothesized that its receptor does not interact with Cry1Aa, and so binding affinity between Cry1Aa and cadherin-like protein which has been regarded as an essential receptor for Cry1As in lepidopteran insects was analyzed. As a result, cadherin-like protein derived from O. scapulalis bound Cry1Aa with high affinity corresponding to that of susceptible insects, suggesting that cadherin-like protein is functional but its function alone cannot induce somatic death. Then we focused on ABC transporter subfamily C2 (ABCC2), a new candidate receptor for cry toxins found as the responsible gene for resistance. According to our recent result, Sf9 cell ectopically expressing Bombyx mori ABCC2 was 10-1000-fold highly susceptive to Cry1As than cadherin-like protein-expressing cell. Therefore, we hypothesized that ABCC2 may be a significant receptor in determining O. scapulalis larvaes' susceptibility to Cry1Aa. Cadherin-like protein or ABCC2 from O.scapulalis ectopically expressed in Sf9 cell and cell susceptibility is now being assessed. We will report this result and discuss the molecule conferring susceptibility or natural resistance to individual insect against Cry toxins.

Keywords:*Cry toxin, ABC transporter*

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Pathology

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The baculovirus uses a stolen host phosphatase to induce enhanced locomotory activity in host caterpillars

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Pathogens are known to usurp or alter the behavior of their hosts for their own benefit. Such behavior modification by animal and even plant viruses is widely observed in insect hosts. One of the earliest documented examples of such behavior modification is Wipfelkrankheit or tree-top disease of caterpillars. A hallmark of this disease is enhanced locomotory activity (ELA) that causes the diseased caterpillars to migrate to the upper foliage of the host plant where they die. We now know that the causative agent of Wipfelkrankheit is a large, double-stranded DNA virus in the family Baculoviridae. Baculoviruses form a large group of arthropod-specific pathogens that commonly attack lepidopteran insects. Two baculovirus genes, protein tyrosine phosphatase (ptp) and ecdysteroid UDP-glucosyltransferase (egt), have been shown to be involved in the induction of ELA such as climbing behavior in baculovirus-infected caterpillars. Here we dissect the functional role that baculovirus PTP plays in the induction of ELA. We surprisingly found that PTP functions as a virus-associated structural protein and not as an enzyme in regard to the induction of ELA. We show that PTP plays a crucial role in virus infection of brain tissues, and hypothesize that this infection results in pathogen control of insect behavior. Since ptp was likely captured from an ancestral host by horizontal gene transfer, our findings tell an amazing story of how the modern baculovirus uses a captured host gene in a completely different way from how it was likely used in the ancestral host.

Keywords:*baculovirus, behavior, evolution*

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Pathology

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Comparative proteomic analysis reveals the mechaminsm of morphological and physiological changes of *Bacillus thuringiensis* YBT-1520 under heat stress and its survival strategies

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Bacillus thuringiensis (Bt) has been widely used for 50 years as a safe biopesticide for controlling agricultural and sanitary insect pests because of its insecticidal crystal proteins. In this study a proteomic approach conbined with physiological investigation was used to describe the proteomic and physiological changes to reveal the survival strategies of Bt YBT-1520 under a long-term heat stress condition (42°C). The changes of the intracellular proteome under heat stress were as follows: (i) some of the heat shock proteins of class I and II were still induced under long-term heat stress; (ii) three non-classical heat shock proteins were also induced under long-term heat stress (iii) 83% of the differentially expressed intracellular proteins decreased (iv) about 50% of the decreased proteins were recovered at the late stage of heat stress condition . Heat stress mainly influenced the characteristics of YBT-1520 on four aspects: (i) the ability of synthesis of ICPs and other potential pathogenic factors were lost or significantly decreased; (ii) cell adhesion and motility were also lost; (iii) the cells did not sporulate, (iv) cells kept accumulating PHB. Proteomic analyses to the physiological changes revealed some strategies of YBT-1520. The ?rst strategy is to up-regulate and down-regulate metabolic enzymes. The second strategy is to increase the synthesis and accumulation of PHB. Under heat stress condition, these strategies would help cells to gain more tolerance to heat stress.

Keywords:*Bacillus thuringiensis, comparative proteomics, heat stress, spore, insecidal crystal proteins, PHB*

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Pathology

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The human gene for Gaucher disease causes the neurodegeneration in the eye of *Drosophila*

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Gaucher disease is caused by defective human glucocerebrosidase. This disease can be classified according to the symptomatic states in 3 types. One has the acute neurodegenetion. However, the molecular mechanism of Gaucher disease is totally unknown. To address the molecular mechanism, we generated Drosophila lines overexpressing human normal and mutated glucocerebrosidase gene (R120W, L444P+A456P+V460V) in the eyes using GMR-GAL4 driver. Three lines of mutated transgenic flies showed defective morphology in Drosophila eyes. Here, we present the data showing a new molecular mechanism of Gaucher disease by using Drosophila model. Furthermore, I talk about the way to prevent the neurodegeneration of Gaucher disease.

Keywords:*Gaucher disease Drosophila*

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Pathology

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Role of the polyhedrin promoter in enhanced locomotory activity induced by baculovirus

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The baculovirus is a classic parasite that alters the behavior or physiology of its host so that progeny transmission is maximized. Baculoviruses do this by inducing enhanced locomotory activity (ELA) that causes the host caterpillars to climb to the upper plant foliage. Recent studies have reported that two baculovirus genes, protein tyrosine phosphatase (ptp) and ecdysteroid UDP-glucosyltransferase (egt), are involved in the induction of this behavior. We have performed locomotion screening assays to find new ELA-associated genes, using Bombyx mori nucleopolyhedrovirus (BmNPV) and its host insect, B. mori. Here we report a novel ELA-associated gene, polyhedrin (polh), which is the most famous baculovirus gene encoding a major structural protein of viral occlusion body. Surprisingly, a polh promoter-defective mutant (polh-proD) did not induce ELA, although neither Polh protein nor polh mRNA is essential for ELA induction. This indicates that the polh promoter region itself is crucial for ELA induction. To assess why polh-proD cannot induce ELA, we next examined the viral propagation in both cultured BmN cells and B. mori larvae. In BmN cells, polh-proD produced budded viruses (BVs) comparable to WT virus, whereas BV titers in the hemolymph of polh-proD-infected larvae were significantly lower than those of WT-infected larvae. In addition, viral gene expression in polh-proD-infected brain remained at a low level even at a late stage of the infection. These results suggest that the polh promoter has a key role in ELA and viral propagation in brain is an important process for ELA induction by baculovirus.

Keywords:*baculovirus, BmNPV, behavior, polyhedrin*

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Pathology

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Host range of new *Bacillus thuringiensis* pest biocontrol agents from Kyrgyzstan

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Naturally occurring entomopathogens are important regulatory factors in insect populations. The most widely used and the most promising microbial control agent is the bacterium Bacillus thuringiensis. To isolate unknown and known BT serovars , the dead and diseased insects and soil samples were collected mainly in pristine habitats of Kyrgyzstan. Isolated natural strains of Bacillus thuringiensis were analyzed by specific primers for Cry I (Lep IF, Lep IR), Cry 3 (CoII F, CoII R) and Cry 4 (Dip IF, Dip IR) crystal endotoxin genes. The results have revealed that a natural isolate 55 S from soil was identified as Bacillus thuringiensis serovar chinensis with two - Cry 3 and Cry I genes, a strain 12-06 isolated from dead leaf beetle was identified as Bacillus thuringiensis serovar pakistani with Cry I-gene, whereas a strain 24.3, isolated from soil was identified as Bacillus thuringiensis IBL 200 with Cry 4 ? genes. We evaluated the efficiency and the host range of isolated B. thuringiensis strains on the larvae stage of pests from Lepidoptera, Hymenoptera and Coleoptera orders. Liquid formulations were compared in laboratory bioassays; larvae were feed on treated plant leaves and followed by efficiency in open field experiments. LC50 and LC95 values for the Bt strains and their formulations show high susceptibility of Helieoverpa armigera Hbn, Lymantria dispar L, Archips rosana L, Leptinotarsa decemlineata S, Leucoma salicis L.,Coliroa cerasi L. The range of host specialization of new natural Bacillus thuringiensis isolates was broad.

Keywords:*Bacillus thuringiensis new isolates, host range, Lepidoptera, Hymenoptera and Coleoptera larvae s*

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Pathology

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Cooperation between two receptors for phagocytosis in defense against bacterial infection in *Drosophila melanogaster*

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Phagocytosis sits at the front line in innate immunity to fight against infection with pathogenic bacteria. In Drosophila, a type of blood cells called plasmatocytes is responsible for the phagocytic elimination of invading bacteria. Plasmatocytes possess two membrane-proteins, Draper and integrin-betanu, and we here examined the possibility that these receptors are involved in the phagocytosis of bacteria. Biochemical and genetic experiments revealed that Draper and integrin-betanu are both required for a maximal level of the phagocytosis of Staphylococcus aureus. A loss of both receptors had severer effects than a loss of either receptor alone on phagocytosis, bacterial growth in flies, and fly death upon infection. A genetic analysis of a role of cell wall components in the susceptibility of S. aureus to phagocytosis showed the importance of lipoteichoic acid and peptidoglycan. Finally, Draper and integrin-betanu bound to lipoteichoic acid and peptidoglycan, respectively. These results indicated that the Draper- and integrin-betanu-mediated phagocytosis of S. aureus take place independently through differential association between phagocytosis receptors and cell wall components. Taken together, Drosophila plasmatocytes are likely to use two receptors for effectively eliminating invading bacteria to survive infectious diseases.

Keywords:*phagocytosis, infection, innate immunity*

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Pathology

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Ribosomal RNA degradation during abortive nucleopolyhedrovirus infection of BM-N cells derived from the silkworm, *Bombyx mori*

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Nucleopolyhedroviruses (NPVs) generally display a high degree of host specificity. Certain NPV-infected insect cells induce anti-viral responses such as apoptosis or global protein synthesis shutdown, resulting in abortive infection. BM-N cells derived from the silkworm, Bombyx mori, are permissive for homologous B. mori NPV (BmNPV), while non-permissive for heterologous NPVs, including Autographa californica multiple NPV (AcMNPV), Hyphantria cunea MNPV (HycuMNPV), Spodoptera exigua MNPV (SeMNPV), and Spodoptera litura MNPV (SpltMNPV). In this study, we demonstrated that severe ribosomal RNA (rRNA) degradation occurred in BM-N cells upon infection with AcMNPV, HycuMNPV, SeMNPV, and SpltMNPV, while no detectable rRNA degradation was observed in BmNPV-infected BM-N cells. rRNA degradation observed in BM-N cells commenced by 4 h pi. Using five lepidopteran cell lines, S. frugiperda Sf9, S. exigua Se301, Sphilosoma imparilis Splm, Lymantria dispar Ld652Y, and B. mori BM-N, and a dipteran cell line Drosophila melanogaster S2, we found that only the BM-N cells exhibited rRNA degradation upon infection with AcMNPV and HycuMNPV. To determine a possible viral protein(s) that was responsible for rRNA degradation in HycuMNPV-infected BM-N cells, we conducted transient expression assays with a cosmid library covering entire HycuMNPV genome and identified HycuMNPV P143 (Hycu-P143) as a viral protein that was responsible for the rRNA degradation. Analyses with plasmids expressing P143 from AcMNPV (Ac-P143), Se-P143, or Splt-P143, showed that these P143 proteins also induced rRNA degradation in BM-N cells. In addition we found that BM-N cells transfected with plasmids expressing Ac-P143, Hycu-P143, Se-P143, and Splt-P143 underwent severe apoptosis.

Keywords:*Baculovirus, Nucleopolyhedrovirus, RNA degradation, P143, BM-N cells*

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Pathological characteristics of *Lymantria dispar* Nucleopolyhedroviruses Isolated from diseased larvae collected In Japan

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The gypsy moth, Lymantria dispar is one of the most important pests of a Japanese larch in Japan. Mitochondrial DNA sequences show that L. dipar is divided into three strains: European strain, Asian strain, and Hokkaido strain. The latter two strains distribute in Japan. L. dipar periodically outbreaks and nucleopolyhedrovirus (NPV) and Entomophaga maimaiga play a role in the downfall of L. dispar populations at the end of outbreaks in Japan. L. dispar NPV (LdNPV) was isolated from diseased larvae collected in Hokkaido (LdNPV-Ho) and in Iwate Prefecture (LdNPV-Iw) in Japan. Those two isolates were evaluated against Gypchek (LdNPV-Am) which is a viral insecticide registered in USA for the gypsy moth control. Restriction endonuclease analysis showed that LdNPV-Ho, LdNPV-Iw and LdNPV-Am were genetically different each other. Droplet feeding bioassays with second instar larvae of the Asian strain and the Hokkaido strain showed that there were no differences in virulence and yield of occlusion bodies among three isolates. Killing time of larvae of the Hokkaido strain infected with LdNPV-Am was significantly longer than that larvae infected with either LdNPV-Ho or LdNPV-Iw. Those results suggested that all three isolates appeared suitable for the control of the Asian and the Hokkaido strains of the gypsy moth.

Keywords:*Nucleopolyhedrovirus, Lymantria dispar, microbial control*

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Pathology

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Molecular responses of non-permissive cell lines to inoculation with *Adoxophyes* nucleopolyhedroviruses (Baculoviridae)

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Baculoviruses often fail to replicate in insect cell lines, even when the cell line was established from the natural host of the virus. In this study, we monitored the molecular responses to three baculoviruses in non-permissive cell lines, and compared them to that in a permissive cell line. A nucleopolyhedrovirus isolated from Adoxophyes honmai larvae (AdhoNPV) was not infectious to the AhL-83 cell line derived from A. honmai (Tortricidae). Another narrow-host-range virus, A. orana NPV (AdorNPV), was also administered to AhL-83 cells, and both AdhoNPV and AdorNPV were further administered to cell lines derived from Homona magnanima (HmL-45; Tortricidae) and Spodoptera frugiperda (Sf-9; Noctuidae). The responses were compared with those to a wider-host-range virus, Autographa californica NPV (AcMNPV). Each cell line was inoculated with one of the three viruses, and we monitored cell growth, viral DNA replication, and transcription of the viral genes ie-1, lef-8, vp39 and polh using PCR and RT-PCR. Occlusion bodies were not produced in any of the cell lines inoculated with AdhoNPV or AdorNPV, and were only observed in Sf-9 cells inoculated with AcMNPV after 48 hpi. Cell growth was not affected in cell lines administered with either AdhoNPV or AdorNPV, but was suppressed within 24 hpi in Sf-9 cells treated with AcMNPV. AcMNPV ie-1 transcription was detected in non-permissive AhL-83 and HmL-45 as well as in permissive Sf9 cells. However, no transcription of any gene tested, and no obvious DNA replication, was detected in any of the three cell lines treated with AdhoNPV or AdorNPV.

Keywords:*nucleopolyhedrovirus, Adoxophyes, non-permissive cell line, PCR, RT-PCR*

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Regulation of *Bombyx mori* initiator caspase Dronc by Hycu-IAP3 of *Hyphantria cunea* nucleopolyhedrovirus

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A number of insect cells infected with nucleopolyhedroviruses (NPVs) undergo apoptosis as an anti-viral response. NPVs encode anti-apoptotic genes, such as Inhibitor of apoptosis (iap), p35 and p49, and suppress virus-induced apoptosis in host cells. Hyphantria cunea MNPV (HycuMNPV) encodes three iap genes, hycu-iap1, hycu-iap2 and hycu-iap3. Only hycu-iap3, but not hycu-iap1 and hycu-iap2, is an indispensable anti-apoptotic gene for productive infection of HycuMNPV in SpIm cells. To get insights into molecular mechanisms underlying apoptosis suppression by Hycu-IAP3, we have studied cellular factors that are involved in the induction of apoptosis. In this study, we cloned and characterized a Bombyx mori homolog of Drosophila initiator caspase Dronc, Bm-Dronc. Bacterially expressed Bm-Dronc preferred synthetic substrates of initiator caspase rather than those of effector caspase. An antibody was raised against the bacterially expressed Bm-Dronc, and changes of endogenous Bm-Dronc in BM-N cells during NPV infection were analyzed. In mock-infected BM-N cells, Bm-Dronc was detected as a full-length form. Apoptosis induction in BM-N cells by infection with p35-defective Autographa californica MNPV (vAcΔp35) promoted the cleavage of Bm-Dronc. To determine whether Hycu-IAP3 affected the endogenous Bm-Dronc, BM-N cells were transfected with hycu-iap3 and then infected with vAcΔp35. Both full-length and cleaved form Bm-Dronc decreased in Hycu-IAP3 expressing cells, indicating that Hycu-IAP3 reduced endogenous Bm-Dronc. This decrease of Bm-Dronc by Hycu-IAP3 was inhibited by a proteasome inhibitor, MG132, suggesting that Hycu-IAP3 promotes degradation of Bm-Dronc through ubiquitin-proteasome pathways.

Keywords:*Nucleopolyhedrovirus, apoptosis, inhibitor of apoptosis, initiator caspase, Bombyx mori*

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Regional expression of the putative virus receptor, nsd-2, and the virus, BmDNV-2 in the midgut

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Bombyx mori densovirus type 2 (BmDNV-2) is a pathogen of flacherie disease in the silkworm. BmDNV-2 replicates only in midgut columnar cells and causes fatal disease. The resistance expressed in some silkworm strains against the virus is determined by a single gene, nsd-2, which is characterized as nonsusceptibility irrespective of the viral dose. Previously we have identified nsd-2 by positional cloning and found that this gene encodes a putative amino acid transporter which may work as a receptor for BmDNV-2. In this study, we investigated the relationship between the BmDNV-2 infection and the part of the midgut expressing nsd-2. RT-PCR analysis with total RNA isolated from anterior, middle, and posterior parts of the midgut showed that nsd-2 was strongly expressed in posterior part of the midgut. In the anterior and middle parts, the expression levels of nsd-2 were very low and not-detected, respectively. On the other hand, the BmDNV-2-derived transcript was clearly detected in all parts of the midgut. There was no significant difference among the parts of the midgut. These results suggest that BmDNV-2 might infect with another mechanism independent to nsd-2 in the middle part of the midgut. There is little information regarding the host's own factors in insects, therefore, we expect that our result will contribute to understanding a novel infection mechanism of insect virus.

Keywords:*Bombyx mori, densovirus, nsd-2, BmDNV-2*

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Molecular dissection of *Bombyx mori* nucleopolyhedrovirus cg30

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The baculovirus cg30 gene is present in the genomes of most Group I and II NPVs. CG30 contains a RING finger motif, which has been shown to be associated with a ubiquitin ligase activity. Also, a previous study in Autographa californica nucleopolyhedrovirus (AcMNPV) revealed that this gene is not essential for virus replication, and a cg30-desrupted virus did not show any significant differences compared with wild-type virus. To know the role of cg30 in other baculoviruses, we constructed a Bombyx mori nucleopolyhedrovirus (BmNPV) mutant (BmCG30D) lacking a functional cg30 by a LacZ cassette insertion, and characterized its infectivity in BmN cells and B. mori larvae. BmCG30D produced fewer budded viruses in BmN cells and occlusion bodies in B. mori larvae as compared with wild-type BmNPV, respectively. Larval bioassays also revealed that CG30 accelerates death of B. mori larvae. These results suggest that CG30 is required for efficient late and very late gene expression. We are currently characterizing two additional mutants, BmCG30CS and BmCG30RingD, both of which likely have no ubiquitin ligase activity. By clarifying the properties of these two mutants in virus-infected cells, we may elucidate the role of CG30-associated ubiquitin ligase activity during baculovirus infection.

Keywords:*BmNPV, cg30, RING finger motif*

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Coexistence of full-length and truncated versions of ecdysteroid UDP-glucosyltransferase gene in the *Antheraea pernyi* nucleopolyhedrovirus isolated from *Samia cynthia ricini*

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The baculovirus ecdysteroid UDP-glucosyltransferase gene (egt) encodes an enzyme catalyzing the sugar conjugation of ecdysteroids to render them inactive. The infected larvae, therefore, have been voracious without moulting and metamorphosis, and produce a large amount of progeny virus. Because of this advantage, egt is conserved among lepidopteran baculoviruses with few exceptions, one of which is the Antheraea pernyi nucleopolyhedrovirus (AnpeNPV) isolated in Liaoning Province, China. Recently, we have found that another AnpeNPV isolated from Samia cynthia ricini in Guangxi Province, China, is a heterogeneous mixture of full-length and truncated versions of egt. Virus clones with truncated egt could not suppress moulting and metamorphosis of infected S. c. ricini larvae, and kill them ca. 5 days faster than a clone with full-length egt, demonstrating that the truncated versions do not encode any functional enzymes. Persistence of the truncated egt in two AnpeNPV populations in China indicates certain advantages of lacking the functional EGT, even with a lesser production of progeny virus. In addition, comparative sequence analysis has revealed that 5' UTR (including the N-terminal coding region), 3' UTR, and a short sequence (28 bp) in the middle of the coding region are conserved among all of the truncated versions, which seem to be transcribed in the infected A. pernyi cell line, suggesting the importance of the conserved regions for the virus. To understand reasons and roles of the truncated egt of AnpeNPV, further investigations of the host-virus interactions are now in progress.

Keywords:*ecdysteroid UDP-glucosyltransferase, baculovirus, Antheraea pernyi, Samia cynthia ricini*

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Pathology

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Clonal variation in the *Helicoverpa armigera* insect cell population

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A total of 16 clones were initially isolated by single-cell cloning from the established HAPO2 cell line derived from pupal ovarian tissue of the cotton bollworm, Helicoverpa armigera. Many of the isolates with highly vacuolated cytoplasms eventually died during subculturing. The 2 rapidly growing clonal cell lines, clones B-5 and D-4 were selected for further analysis. The clonal cell lines differed in their predominant morphology, and relative ability to support replication of the H. armigera nucleopolyhedrovirus (HearNPV). The B-5 and D-4 clonal cell lines were permissive to HearNPV exhibiting nuclear hypertrophy and occlusion bodies (OB) formation. However, the D-4 cell line was more susceptible to HearNPV infection than B-5 cell line, presenting higher infection level and high number of OB produced per cell. At late stages of infection, cell lysis and release of OB into the culture medium occurred in HearNPV-infected HAPO2 D-4 cells. The data described in this study indicate that clone D-4 will be a highly productive new clonal cell line that will be useful for the production of baculoviruses, recombinant protein expression in the baculovirus/insect cell system and other applications in biotechnology.

Keywords:*clonal cell line, insect cell, Helicoverpa armigera, nucleopolyhedrovirus*

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Pathology

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Isolation and characterization of entomopathogenic fungi isolated from wild mosquito

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Mosquito vector control is an important part of controlling infectious diseases. Although the use of chemical insecticides is the mainstay for disease vector control, development of insecticide resistance has been reported. Now new vector control approaches are required. So, the aim of this study was to search promising isolates as fungal biopesticide of vector mosquito. Initially, entomopathogenic fungi were isolated from wild mosquito collected in Japan and Burkina Faso by two means of isolation methods (using the whole body of mosquito and the mosquito sterilized body surface), and molecular-based identification was conducted. As a result, Beauveria bassiana, B. brongniartii, Isaria farinosa, I. fumosorosea, Paecilomyces carneus, P. lilacinus, Lecanicillium araneicola, Lecanicillium spp., Simplicillium lamelicola and S. lanosoniveum were detected. Subsequently, these isolates were applied to bioassay against adult female Anopheles stephensi. Twenty seven isolates which showed higher pathogenicity and/or infectivity in preliminary experiment were evaluated the 50 % lethal time (LT50) and infection rate. As a result, LT50 values were ranged from 5.8 days to 12.5 days in isolates from whole mosquito body, and 6.6 days to 14.9 days in isolates from inside of the mosquito body, while, control plot was 16.6 days. Isolates from inside of the mosquito body showed tendency to lower virulence than isolates from whole mosquito body. Especially, Beauveria bassiana 60-2 showed highest virulence and infection rate against An. stephensi. So, our results suggested that wild adult mosquitoes have various entomopathogenic fungi that might have the potential for controlling vector mosquito as a biopesticide.

Keywords:*entomopathogenic fungi, Beauveria bassiana, mosquitoes, vector control, Anopheles, fungal isolation*

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PS5TH196

Pathology

P5

The large pine weevil (*Hylobius abietis*) as a vector of saprophytic fungi

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Saprophytic fungi Heterobasidion parviporum Niemela & Korhonen and H. annosum (Fr.) Bref are recognized as a major pathogenic saprophytic fungi of both young seedlings and mature trees, where they produce characteristic root and butt rot. Phlebiopsis gigantea (Fr.) Julich is a common saprophytic fungus that causes white rot of conifer logs and stumps. It is used as a biological control of annosum root rot, caused by Heterobasidion spp. The aim of this study was to investigate the large pine weevil (Hylobius abietis L.), an important pest of transplanted coniferous seedlings, capability to preserve alive in digestive tract the mycelium of fungi. In laboratory tests the mycelium of P. gigantea survived the most in viable condition through the digestive tract of the large pine weevil. In additional in pine weevils excrements were identified also the fungi of H. parvivorum. The vector insects were both males and females. Experiments showed that the large pine weevil could be the vector of pathogens Heterobasidion spp. and also for their natural enemy P. gigantea. The research was supported by the Estonian Science Foundation (grant numbers 6781 and 9449).

Keywords:*Hylobius abietis, Heterobasidion spp., Phlebiopsis gigantea, saprophytic fungi, pest, root rot*

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PS5TH197

Pathology

P5

Genome and transcriptome-wide analysis of the immune-related genes in the brown planthopper *Nilaparvata lugens*

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The brown planthopper (Nilaparvata lugens) is one of the most serious rice plant pests in Asia. BPH causes extensive rice damage by sucking rice phloem sap, which results in stunted plant growth and transmission of plant viruses. Little is known about the immunological mechanisms occurring in N. lugens. In this study, we performed the genome- and transcriptome-wide analysis of the immune-related genes via the next-generation high-throughput DNA sequencing technology. We identified a large number of genes encoding N. lugens pattern recognition proteins, modulation proteins, effectors and the molecules involved in the immune signaling pathways including Toll, Imd, JAK-STAT and JNK pathways. We investigated the gene expression profiles responded to the bacterial and viral infection at the transcript levels.

Keywords:*Nilaparvata lugens, immune-related genes*

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PS5TH198

Pathology

P5

Identification of a new *Bombyx mori* nucleopolyhedrovirus strains and analysis of its bro gene family

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The highly pathogenic Bombyx mori nucleopolyhedrovirus (BmNPV) has caused severe damages to sericulture in many countries, and the relationship between the pathogenicity of various BmNPV strains and their geographical evolution has been the topic of our interest. In this study, we isolated a new BmNPV strain from Thailand (BmNPV-Thai), based on the sequences of its conservative genes p10, p35, polh, egt and vp39. The obtained BmNPV appeared to have its baculovirus repeated ORF (bro) genes different from four other well-known BmNPV strains of China (GD, CQ1), Japan (T3) and France (SC7). Specifically, BmNPV-Thai has only the regions of bro-a, bro-c and bro-d, but not bro-b and bro-e genes. These bro genes of BmNPV are only localized in two subgroups which high homology to their counterparts and the encode BRO proteins differ mainly at their N-terminal amino acid residues. Phylogenetic analysis indicates that the evolution of the bro genes of the five BmNPV strains is not obviously associated with their geographic locations.

Keywords:*Bombyx mori nucleopolyhedrovirus, Strain Thai, p10, p35, polh, egt, vp39, bro, identification*

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PS5TH199

Pathology

P5

Isolation of *Bacillus thuringiensis* from forest environments in Korea

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We investigated the distribution, toxicity, morphology, and protein profiles of Bacillus thuringiensis isolates from forests in Korea to isolate naturally occurring novel B. thuringiensis. A total of 170 B. thuringiensis isolates were obtained from 832 samples producing spore and parasporal inclusion bodies. In toxicity tests for lepidopteran, dipteran, and coleopteran insects, 57.6% isolates were toxic only to Lepidoptera, 5.3% were toxic only to Diptera, and 24.1% were toxic to both Diptera and Lepidoptera. The remaining collections (13.0%) were not toxic to the tested insects. The shapes of the parasporal crystals produced in B. thuringiensis isolates were bipyramidal, spherical, ovoid, or irregular. As their toxicities varied with parasporal crystal shape, B. thuringiensis isolates possessing bipyramidal or irregular parasporal crystals were largely toxic to lepidopteran species whereas those producing spherical parasporal crystals were mainly toxic to dipteran species. B. thuringiensis toxic to both dipteran and lepidopteran insects contained 130- and 70-kDa parasporal crystals, whereas B. thuringiensis toxic to lepidopteran insects expressed 130-kDa parasporal crystals. The results suggest that forest areas in Korea are a rich source of B. thuringiensis and need to be further explored to discover novel B. thuringiensis isolates.

Keywords:*Bacillus thuringiensis, Microbial control, Natural resources, Parasporal crystal*

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PS5TH200

Pathology

P5

Migrations and multiplications of three kinds of pine wood nematodes in *Pinus thunbergii*

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To ascertain the effects of migration and multiplication of pine wood nematodes (PWNs) in susceptible pine trees on pine wilt disease, pathogenic Bursaphelenchus xylophilus and non-pathogenic East Asian type and European type of B. mucronatus were inoculated on top cross-cut surfaces of 1-year-old stem cuttings of 5-year-old Pinus thunbergii seedlings. One thousand PWNs were inoculated on 20 cm pine stem cuttings and then the stem cuttings were cut into 2.5 cm segments on 1 and 5 days after inoculation and PWNs were extracted from each 2.5 cm segments to investigate distributions of pine wood nematodes. Also 200 PWNs were inoculated on 20 cm pine stem cuttings and Botrytis cinerea fungal mats and then they were extracted on 7 days after inoculation to measure increases of three kinds of pine wood nematode population. The distributions of pathogenic PWN of B. xylophilus in 20 cm pine stem cuttings were not significantly different from those of non-pathogenic PWNs of East Asian type and European type of B. mucronatus on 1 and 5 days after inoculation. The population growth rate of B. xylophilus on B. cinerea, however, was significantly higher than those of East Asian type and European type of B. mucronatus and the mean number of B. xylophilus remained in pine stem cuttings was also significantly higher than those of the other two PWNs. Thus pathogenicity of B. xylophilus is closely related to the multiplying ability rather than the migration ability compared with non-pathogenic East Asian type and European type of B. mucronatus.

Keywords:*pine wilt disease, migration, multiplication, pine wood nematode, Pinus thunbergii*

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PS5TH201

Pathology

P5

High-throughput expression of recombinant protein using baculovirus

Jae Young Choi[1], Yang-Su Kim[2], Yong Wang[3], Xue Ying Tao[4], Qin Liu[5], Jong Yul Roh[6], Soo Dong Woo[7], Byung Rae Jin[8], Yeon Ho Je[9]

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Recently, we constructed a novel recombinant baculovirus genome, bEasyBac, enabling easy and fast generation of pure recombinant baculovirus without any purification step. In the bEasyBac, bacteriophage lambda site-specific attachment (att) sites were introduced to facilitate the generation of recombinant viral genome by in vitro transposition. Moreover, extracellular RNase gene from Bacillus amyloliquefaciens, barnase, was expressed under the control of Cotesia plutellae bracovirus (CpBV) ORF3005 early promoter to negatively select against non-recombinant background. The bEasyBac could replicate in host insect cells only when the barnase gene was replaced to gene of interest by in vitro transposition. Insect cells transfected with the bEasyBac not only showed slightly cytopathic effects with reduced cell growth after transfection but also did not support any viral replication. When the bEasyBac was transposed with pDualBac-EGFP and the EGFP expression efficiency along passage was investigated, the resulting recombinant virus, EasyBac-EGFP, showed comparable level of EGFP expression efficiency with the plaque-purified recombinant virus, AcEGFP, which was constructed using bAcGOZA system, whereas, the non-purified AcEGFP showed quite reduced level of EGFP along passages. Moreover, no non-recombinant backgrounds were detected from unpurified EasyBac-EGFP stocks. Based on these results, high-throughput condition for generation of multiple recombinant viruses in a time was established. These results suggest that the bEasyBac has an effective benefit enabling for high-throughput baculovirus expression vector without purifying recombinant virus.

Keywords:*baculovirus expression system, EasyBac, in vitro transposition, barnase, high-throughput*

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Pathology

P5

Molecular characterization of a *Bacillus thuringiensis* serovar mogi (flagellar serotype 3a3b3d) with mosquitocidal activity

Qin Liu[1], Jong Yul Roh[2], Jae Young Choi[3], Yong Wang[4], Xue Ying Tao[5], Joo Hyun Lee[6], Song Eun Kim[7], Zhenli Fu[8], Jae Su Kim[9], Yeon Ho Je[10]

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Plasmids from Bacillus thuringiensis have been implicated in pathogenicity as they carry the genes responsible for different types of diseases in mammals and insects. B. thuringiensis serovar mogi of a novel serogroup (H3a3b3d), which showed mosquitocidal activity against Anopheles sinensis and Culex pipiens pallens, was isolated from fallen leaves in Mungyeong city, Republic of Korea. In contrast to the complicated plasmid profiles of B. thuringiensis H3 serotype strains, the B. thuringiensis serovar mogi contained only megaplasmid (> 30 MDa) on which the toxin genes were occasionally located. Sequence analysis using 454-pyrosequencing revealed that the megaplasmid harbored at least seven putative cry genes, showing about 84%, 75%, 73%, 58%, 84%, 39% and 75% similarities in amino acid sequences with Cry27Aa, Cry19Ba, Cry20-like, Cry56Aa, Cry39ORF2, Cry8Ba and Cry40ORF2, respectively. These cry genes were cloned to the Escherichia coli-B. thuringiensis shuttle vector, pHT1K, and then introduced into an acrystalliferous B. thuringiensis Cry-B strain for further molecular characterization. This novel 3a3b3d type strain, B. thuringiensis serovar mogi, could be used as a good resource for studying unknown mosquitocidal cry genes.

Keywords:*Bacillus thuringiensis, novel serotype, H3a3b3d, megaplasmid, cry gene, mosquitocidal activity*

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Pathology

P5

Functional characterization of *Autographa californica* multiple nucleopolyhedrovirus ORF43

Xue Ying Tao[1], Jae Young Choi[2], Yong Wang[3], Jong Yul Roh[4], Joo Hyun Lee[5], Qin Liu[6], Jong Bin Park[7], Jae Su Kim[8], Soo Dong Woo[9], Byung Rae Jin[10], Yeon Ho Je[11]

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ORF43 (ac43) of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is a highly conserved baculovirus gene whose function is unknown. To determine the role of ac43 in baculovirus life cycle, we constructed an ac43-deleted mutant AcMNPV, Ac43KO. After transfection into Spodoptera frugiperda cells, Ac43KO produced polyhedra with much larger size compared to those of wild-type AcMNPV. Interestingly, some of nucleocapsids were singly enveloped in the polyhedrin matrix while the nucleocapsids of AcMNPV have been known to be multiply enveloped. Furthermore, Ac43KO led to a defect in transcription and expression of polyhedrin, which resulted in less occlusion body (OB) production. However, Ac43KO did not affect production of budded virus (BV) since there was no remarkable difference in BV titer. These results suggest that ac43 plays an important role in expression of polyhedrin, morphogenesis of OB and assembly of virions occluded in OB.

Keywords:*AcMNPV, ac43, knockout mutant, OB morphogenesis, nucleocapsid packaging*

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Pathology

P5

Novel baculovirus expression vector enabling simple purification of recombinant protein using polyhedrin fusion

Jong Yul Roh[1], Jae Young Choi[2], Joong Nam Kang[3], Yong Wang[4], Hee Jin Shim[5], Qin Liu[6], Xue Ying Tao[7], Hong Guang Xu[8], Soo Dong Woo[9], Byung Rae Jin[10], Yeon Ho Je[11]

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Previously, we found that expression by translational fusion of the polyhedrin (Polh)-green fluorescence protein (GFP) led to the formation of granular structures and these fluorescent granules were easily precipitated by high-speed centrifugation. Here, we developed an easy, fast, and mass purification system using this baculovirus expression system (BES). An enhanced GFP (EGFP) fused with Polh gene at the N-terminus including an adaptor and enterokinase (EK) site between Polh and EGFP was expressed in Sf9 cells. The cells infected by AcPolhEKA-EGFP produced fluorescent granules. The EGFP fusion protein was purified from granule-containing cells according to three steps; cell harvest, sonication and EK digestion. Through the final enterokinase digestion, EGFP was presented mainly in the supernatant (93.3%) and the supernatant also showed a pure EGFP band. These results suggest that the combined procedure of Polh fusion expression and enterokinase digestion can used for the rapid and easy purification of other proteins.

Keywords:*polyhedrin fusion, granulation, EGFP, baculovirus expression system, easy purification system*

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PS5TH205

Pathology

P5

Cloning of circular DNAs from microorganisms using a novel plasmid capture system

Jong Yul Roh[1], Yong Wang[2], Qin Liu[3], Xue Ying Tao[4], Jae Young Choi[5], Hee Jin Shim[6], Hong Guang Xu[7], Soo Dong Woo[8], Byung Rae Jin[9], Yeon Ho Je[10]

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Plasmid capture system (PCS) was developed for easy cloning and manipulation of circular double-stranded DNA from various sources. Recently, we improved PCS system (named PCS-LZ) to clone relatively large-sized DNA molecules (30~150 kb). PCS-LZ donor consists of a Mini-F replicon and a kanamycin resistance marker between Tn7L and Tn7R regions. Both replicon and marker gene of PCS-LZ donor are transferred into target plasmid DNAs by in vitro transposition and the transposed DNAs can replicate in E. coli cells by transformation. White/blue screening by LacZ expression is also available to avoid backgrounds. Up to now, we acquired various circular DNA clones from four sources such as plasmids of B. thuringiensis, bacteriophage genome isolated from B. thuringiensis, genome segments of Cotesia glomerata bracovirus, and polymorphic genomes of Autographa californica nucleopolyhedrovirus. Conclusively, we expect this PCS-LZ system would be a powerful tool for genomic analysis and mutagenesis study at the area of invertebrate pathology and further its application will be enlarged to the vertebrate pathology area.

Keywords:*plasmid capture system (PCS), PCS-LZ, in vitro transposition, circular DNA cloning, Bacillus thuringiensis, CgBV, AcMNPV*

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PS5TH206

Pathology

P5

A Novel, highly effective and environmentally friendly recombinant baculovirus insecticide, NeuroBactrus

Hee Jin Shim[1], Jae Young Choi[2], Yong Wang[3], Xue Ying Tao[4], Qin Liu[5], Jong Yul Roh[6], Jae Su Kim[7], Soo Dong Woo[8], Byung Rae Jin[9], Yeon Ho Je[10]

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A novel recombinant baculovirus, NeuroBactrus, was constructed to develop an improved baculovirus insecticide with additional beneficial properties, such as a higher insecticidal activity and improved recovery, compared to wild-type baculovirus. For the construction of the NeuroBactrus, the Bacillus thuringiensis cry1-5 crystal protein gene was introduced into the Autographa californica nucleopolyhedrovirus (AcMNPV) genome by fusion of polyhedrin-cry1-5-polyhedrin under the control of the polyhedrin promoter. In the opposite direction, an insect-specific neurotoxin gene, AaIT, from Androctonus australis was introduced under the control of an early promoter from Cotesia plutellae bracovirus by fusion of a partial fragment of orf603. The Polyhedrin-Cry1-5-Polyhedrin fusion protein expressed by the NeuroBactrus was not only occluded into the polyhedra, but it was also activated by treatment with trypsin, resulting in an approximately 65-kDa active toxin. In addition, qrt-PCR revealed that the neurotoxin was expressed from the early phase of infection. The NeuroBactrus showed a high level of insecticidal activity against Plutella xylostella larvae and a significant reduction in the median lethal time (LT50) against Spodoptera exigua larvae compared to those of wild-type AcMNPV. Re-recombinant mutants derived from NeuroBactrus in which AaIT and/or cry1-5 were deleted were generated by serial passages in vitro. Expression of the foreign proteins (Bt toxin and AaIT) was continuously reduced during the serial passage of the NeuroBactrus. Moreover, polyhedra collected from S. exigua larvae infected with the serially passed NeuroBactrus showed insecticidal activity similar to that of wild-type AcMNPV. These results suggested that the NeuroBactrus could be recovered to wild-type AcMNPV through serial passaging.

Keywords:*recombinant baculovirus, insecticide, Bacillus thuringiensis, AaIT, recovery to wild-type*

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Pathology

P5

Incidence of entomopathogenic fungi from agricultural and forest areas and their pathogenicity in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in Southern Thailand

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Southern Thailand located on the Malay Peninsula is rich of fauna and flora in nature. Diversity of microorganisms concerning microbial control of insect pests is also well off. Therefore, an initial research project was designed to collect and isolate entomopathogenic fungi from soil and insect cadavers in areas of agriculture and forest. Sixty-six isolates of entomopathogenic fungi were obtained comprising 64 isolates of Metarhizium anisopliae and two isolates of Beauveria bassiana. Fifty eight were isolated from soil and eight from insect cadavers (six isolates of M. anisopliae and two of B. bassiana). The soil samples from Songkhla province gave the highest number of entomopathogenic fungi (39 isolates) followed by the soil samples from Patthalung province (14 isolates). In contrast, the soil samples from Nakhon Si Thammarat province had the lowest number (5 isolates). The entomopathogenic fungi were divided into 4 groups according to their mycelial growth and sporulation. Later the isolated entomopathogenic fungi were assessed for their pathogenicity in adult fruit fly, Bactrocera cucurbitae (Coquillett). Each isolates caused significantly different degrees of virulence on the flies, with M. anisopliae isolate PSUM05 giving 100% mortality of adult male and female within 5 and 7 days. Extending observation up to 15 days, although B. bassiana could induce mortality of the adult flies, mortality in the flies was less than 56%.

Keywords:*entomopathogenic fungi, Metarhizium anisopliae, Beauveria bassiana, pathogenicity*

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PS5TH208

Pathology

P5

Real-time tracing of AcMNPV infection to Plutella xylostella and the interaction between virus and parasitoid

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Baculovirus is an important microorganism for controlling insect pests in the field, and is also crucial for integrating into an ecologically sound IPM program. In order to trace baculovirus infection, a recombinant baculovirus, vAcGFP, derived from Autographa californica nucleopolyhedrovirus (AcMNPV) was constructed. This virus contains a wt polyhedrin gene and a GFP coding region driven by p10 promoter. The LC50 values of vAcGFP and wt AcMNPV to the 3rd instar larvae with the diet incorporation method were 34.4 and 25.6 OBs/mm3 of diet, respectively. The LT50 values of these two viruses were relatively close. The larvae infected with vAcGFP were easily detectable due to the emittion of green fluorescence (505nm) as irradiated by a 365nm UV light. Its FT50 (50% fluorescense-emitting time) was 65 h earlier than LT50 at 763 OBs/mm3. The 3rd instar larvae of P. xylostella were inoculated with vAcGFP and simultaneously parasitized by the parasitoid, Cotesia plutellae. A fluorescence-emitting rate of 84 and 32% was separately coupled with 12 and 54% cocooning rate at 76.3 and 7.6 PIBs/mm3, respectively, and only 5~8% cocoons with fluorescence was detected. The cocooning rate of C. plutellae was decreasing through the time of parasitism and the increasing of viral concentration. In conclusion, GFP could be used as a specific and non-destroying biomarker for detection of insect dynamics after being infected with NPV in the field. In addition, our study shed a light for further study of the complicated interactions between baculovirus and broconid wasp in an insect host.

Keywords:*Autographa californica nucleopolyhedrovirus (AcMNPV), green fluorescence protein (GFP), recombinamt virus, Plutella xylostella, Cotesia plutellae*

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PS5TH209

Pathology

P5

Synergistic interaction of *Beauveria bassiana* and *Bacillus thuringiensis* for the control of tomato fruitworm *Helicoverpa armigera*

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Toxicity of commercial formulation of Bacillus thuringiensis and Beauveria bassiana was evaluated against second instar larvae of tomato fruitworm Helicoverpa armigera collected from different major tomato growing districts in Punjab, Pakistan viz-a-viz Khanewal, Multan, Muzaffar Garh, Bahawalpur and Pakpattan. Larval development was greatly reduced when toxin concentration in diet increased from lower to higher concentration. The mortality increased with increase in application rate of either B. bassiana or Bt while significantly high mortality (100.00±0.00) was recorded in Pakpattan population for the combined use of Bt and B. bassiana. Combination of lower dose of B. bassiana (1×106 conidia/ml) with both concentrations of Bt showed additive effect on mortality of H. armigera while higher dose of B. bassiana (1×107 conidia/ml) synergistically enhanced mortality in all localities. Lowest pupation (0.00±0.00) rate was observed in simultaneous application of higher concentration of Bt and B. bassiana (1×107spores/ml+1µg/g Bt) which showed highest mortal effect on larvae of H. armigera. Rate of mycosis was predominantly high in larvae found dead in individual application of B. bassiana and certainly no salmon color change was found in larvae died with B. thuringiensis.

Keywords:*Tomato fruitworm, Bacillus thuringiensis, Beauveria bassiana, Synergism*

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PS5TH210

Pathology

P5

Entomopathogenic fungi: An alternative in the control of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)?

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Iranian Research Institute of Plant Protection, Iran

The Asian citrus psyllid, Diaphorina citri Kuwayama, is a vector of Candidatus Liberibacter asiaticus which causes citrus greening disease. This has become the most damaging disease of citrus trees In Iran since its first incidence in 2007. Controlling the vector is the most efficient approach for controlling this disease. Entomopathogenic fungi can be useful alternatives to control the vector. Two fungal isolates, Metarrhizium anisopliae ('DEMI 002') and Paecilomyces tenuipes ('DEPI 002') were assessed against adult psyllids in the laboratory. To study their mortalities and the effect of sprayed area on mortality, a detached-leaf bioassay was used. Four leaf sections were placed together in a Petri dish and one to four of them were sprayed by the fungal suspensions at a concentration of 7×105 spores/ml. A single adult was released into each Petri dish (27±2 ?C, 75% RH). Five days after treatment, 'DEMI 002' and 'DEPI 002' caused 71% and 29% mortalities, respectively. 'DEMI 002' was significantly more effective ($F = 6.88$; $df = 4, 23$; $P < 0.05$) than 'DEPI 002'; However, there was no statistical difference between different leaf area coverage. More studies under greenhouse and field conditions are required.

Keywords:*Diaphorina citri, citrus greening disease, Metarrhizium, Paecilomyces, mortality*

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PS5TH211

Pathology

P5

Antimicrobial and antioxidant activity in liquid culture filtrates of entomopathogenic fungi

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The entomopathogenic fungi were an important natural pathogenic of insects that has been developed as potential biological control agents for many important agricultural, forest and medical pests. Several these fungi produce a wide range of secondary metabolites with high therapeutic value as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor and repellent. Therefore, this study was performed to select the antibacterial against two pathogenic bacteria including Ralstonia solanacearum and Escherichia coli and antioxidant activity of liquid culture filtrates of 347 entomopathogenic fungi form Korea soils using novel method which represents a quick and easily applicable tool obtaining large number of samples. As results, seventy-two isolates (20%), twenty-two isolates (6%) and twenty-two isolates (6%) of these fungal metabolites produced anti-R. solanacearum, anti-E. coli and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals scavenging activity compounds, respectively. The preferential antibacterial and radical scavenging activity gives evidence that these entomopathogenic fungal metabolites might be useful as a source for bacteria control and pharmaceutical interest

Keywords:*entomopathogenic fungi, antimicrobial activity, antioxidant activity*

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PS5TH212

Pathology

P5

Genomic sequence analysis of the *Hyphantria cunea* granulovirus isolated in Korea

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Hyphantria cunea is a fall webworm is considered an agricultural pest. It is a major pest of many board-leaved trees. To better understand Hyphantria cunea granulovirus (HcGV), the nucleotide sequence of the HcGV DNA genome was determined and analyzed. HcGV isolated from naturally infected Hyphantria cunea larvae in Korea. The full genome sequence of HcGV were determined, analyzed and compared to those of other baculoviruses. The entire nucleotide sequence of the HcGV genome was fully sequenced using 454 pyrosequencing. The HcGV genome consisted of 114,557 bp and had on overall G+C content of 39.30%. Computer- assisted analysis predicted 130 open reading frames (ORFs) of 50 amino acids or greater that showed minimal overlap. HcGV shares more than 106 ORFs with other baculoviruses. The positions of at least some baculovirus homologous repeat regions appear to be conserved relative to specific baculovirus genes. The gene parity plot analysis, percent identity of the gene homologues and a phylogenetic analysis suggested that HcGV is betabaculovirus that is closely related to Xestia c-nigrum granulovirus but with a highly distinct genomic organization.

Keywords:*HcGV, Hyphantria cunea, 454 pyrosequencing*

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PS5TH213

Pathology

P5

Genetic expression characteristics of other nematodes cellulase-related genes except for *Bursaphelenchus xylophilus*.

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Bursaphelenchus xylophilus(Pine wood nematode, PWN) has been recognized as a casual pathogen of pine wilt disease (PWD). Bursaphelenchus xylophilus induce PWD as feed on plant cells ? consist of cellulose as parenchyma tracheids. Pine wilt disease is the one of serious forest disease in Korea so that conducted various mechanical and chemical researches for control damages, but insufficient as biotechnical researches. Some researches have suggested that Bursaphelenchus xylophilus secretes highly active cellulase in order to break down the cell walls and cell membranes of parenchyma tracheids. The results of previous studies have indicated that the cellulase of PWN performs a crucial function in these pathogenic substrances. Other nematodes as Busaphelenchus genus have speculated taken cellulase but not developed yet except for PWN. If other nematodes cellulase characteristics develop biotechnically except for PWN, contribute to research of Bursaphelenchus have been controversies about pathogenic. Therefore nematodes as Busaphelenchus genus ? Bursaphelenchus xylophilus, B. mucronatus(East asian type), B. mucronatus(European type), B. doui, B. thailadae, B. hylobianum, B. tusciae, B. yongensis ? grown on a lawn of Botrytis cinerea cultured on PDA media, extracted total RNA and synthesized cDNA. PWD cellulose related gene screened by NCBI (http://www.ncbi.nlm.nih.gov/) ? ACD12136.1, BAD34543.1, BAD34544.1, BAD34545.1, BAD34546.1, ACM44321.1, ACM44322.1, ACM44323.1 and compared with each other. Primers designed different site of the conserved domain after multiple sequence alignment and genetically compared with 8 Bursaphelenchus by RT-PCR.

Keywords:*Bursaphelenchus, cellulase*

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Pathology

P5

Sequence analysis and characterization of adaptor protein MyD88 from a coleopteran beetle, *Tenebrio molitor*

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MyD88 considered as an adaptor protein forms a major component of the Toll pathway during the response to fungal and Gram-positive bacterial infections and is recruited by the cleaved Spatzle-Toll complex. This is because; the Toll family in insect models studied and a similar family of Toll-like receptors (TLRs) identified in mammals signals to NF-κB through the adaptor, MyD88 that binds to the kinase Pelle in degrading Cactus, inducing the translocation of transcription factors, Dif and Dorsal, thus inducing the effector molecules. In an attempt to study the involvement of MyD88 as an integrator of signals from Toll, the full length cDNA of MyD88 have been cloned from the coleopteran beetle, Tenebrio molitor. Full-length sequence of MyD88 annotated by DNASTAR program reveals an open reading frame of 1172 bp encoding 391 amino acids. It has a 5'- and 3'-untranslated region of 83 bp and 300 bp respectively with polyA-tail comprising of 19bp starting from nucleotide no. 1557. BLAST and Multiple Alignment results have been conducted on MyD88 protein sequence, and it has been observed that it has significant sequence similarity with MyD88 of Tribolium castaneum. Putative conserved domains include an NH2-terminal death domain and COOH-terminal Toll-IL-1R domains that are implicated in interacting with Toll receptor family. The C-terminal sequences of MyD88 from closely-related groups indicate the uniqueness and less-conserved nature, a potent strategy for future development of antibodies with high-end specificity.

Keywords:*MyD88, Toll-Signaling, Sequence analysis, Tenebrio molitor*

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Pathology

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Cloning and expression pattern of a galectin homologue from a coleopteran insect, *Tenebrio molitor*

Hyo Jeong Lee[1], Gi Won Seo[2], Jeong Hwan Seong[3], Dong Hyun Kim[4], Seongmin Kang[5], Yong Hun Jo[6], Yong Seok Lee[7], Bok-Leul Lee[8], Nam Jeong Kim[9], Yeon soo Han[10]

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Galectin has been known to play a key role in insect innate immunity and pathogen recognition process. From the expressed sequence tags (ESTs) of T. molitor larvae, we have identified a galectin homologue (TmGalectin). The cDNA contained a 3′ untranslated region of 165 nucleotides, including a stop codon (TAA) and a poly (A) tail although 5'-UTR is not currently available. It consists of 660 bp with an open reading frame of 495 bp, encoding 164 amino acids. The deduced amino acid sequence of TmGalectin has relatively low identities (50%) to various insect galectin. Developmental expression analysis shows that TmGalectin is highly expressed during the pupal stages (day 2 and 5). Most interestingly, TmGalectin mRNA is highly expressed in adult integument and gut. Furthermore, TmGalectin mRNA was highly induced after immune-challenging with various immune elicitors such as Lys/DAP-type peptidoglycan (PGN), Beta 1,3 glucan, and Acholeplasma lyaste. These data suggests that TmGalectin may play a role in innate immune responses although it remains to further elucidate the precise biological functions in T. molitor.

Keywords:*galectin, innate immunity, Tenebrio*

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Pathology

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Cloning and in silico analysis of spatzle 7 homologue from a coleopteran insect, *Tenebrio molitor*

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Spatzle has been known to play a key role in Toll signaling cascade in response to gram-positive bacterial and fungal infection in insects. From the expressed sequence tags (ESTs) of T. molitor larvae, we have identified a spaztle homologue (TmSpatzle7). The cDNA contained a 3′ untranslated region of 112 nucleotides, including a stop codon (TAA) and a poly (A) tail although 5'-UTR is not currently available. It consists of 742 bp with an open reading frame of 630 bp, encoding 209 amino acids. The deduced amino acid sequence of TmSpatzle7 has relatively low identities (50%) to Tribolium castaneum Spatzle7, and also with T. castaneum spaetzle 1B (28%) and Aedes aegypti sp. Currently, TmSpaztle7 is expressed in pET expression vector to isolate recombinant TmPGRP-LE. Since spaztle 7 has not been fully characterized until now, expression and functional analysis will be conducted in response to various immune elictors and it still remains to further elucidate the precise biological functions of TmSpatzle7 in T. molitor.

Keywords:*Spatzle, Tenebrio, Toll*

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Pathology

P5

Identification and partial characterization of PGRP-LE homologue from a coleopteran insect, *Tenebrio molitor*

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Peptidoglcan recogniatioin protein-LE (PGRP-LE) plays a key role in immune deficiency (IMD) signaling cascade in response to gram-negative bacterial infection in insects including Drosophila and Tenebrio. From the expressed sequence tags (ESTs) of T. molitor larvae, we have identified a PGRP-LE homologue (TmPGRP-LE). The cDNA contained a 5′ untranslated region of 116 nucleotides and a 3′ untranslated region of 72 nucleotides, including a stop codon (TAA) and a poly (A) tail. It consists of 1,181 bp with an open reading frame of 993 bp, encoding 330 amino acids. The deduced amino acid sequence of TmPGRP-LE has relatively low identities (68%) to Tribolium castaneum PGRP-LE. Currently, TmPGRP-LE is expressed in pET expression vector to isolate recombinant TmPGRP-LE. Expression analysis will be conducted in response to various immune elictors and it still remains to further elucidate the precise biological functions of TmPGRP-LE in T. molitor.

Keywords:*PGRP-LE, Tenebrio, Innate Immunity*

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Pathology

P5

Overexpression and purification of the recombinant serralysin-like metalloproteases in *Escherichia coli*..

Kosuke Kawano[1], Kazuhiro Iiyama[2], Jae Man Lee[3], Takahiro Kusakabe[4], Chisa Yasunaga-Aoki[5], Susumu Shimizu[6]

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Proteases are found in a wide range of organisms including bacteria, fungi and so on. Some of them were identified as virulence factors and have important roles in pathogenesis. In our previous work, Serratia liquefaciens Kuo1-1 isolated from ant lion larvae was highly virulent to two insect species, the silkworm, Bombyx mori and the American cockroach, Periplaneta americana. It was demonstrated that S. liquefaciens FK01, spontaneous a nalidixic acid-resistant mutant of Kuo1-1, produced two proteases. The proteases were determined to be metalloproteases upon inhibition test with specific metalloproteinase inhibitor EDTA. Zymography revealed that molecular masses were approximately 50 kDa. These results indicated that these proteases belonged to serralysin family. The genes encoding the proteases were cloned and designated ser1 and ser2. Conserved regions including a zinc binding motif (HEXXHXUGUXH), glycine-rich repeats (GGXGXD) and an ABC exporter motif (DXXX) were found in both Ser1 and Ser2 encoded by ser1 and ser2, respectively. To ascertain the toxicity to insects, recombinant proteins were produced in Escherichia coli BL21 DE3 (pLysS). The region corresponding to the putative mature proteins were amplified by PCR. The amplicons were cloned into the IPTG-inducible pET16b by In-Fusion technology. The recombinant proteins (rSer1 and rSer2) induced by IPTG were observed in insoluble inclusion bodies. The insoluble recombinant proteins were solubilized by 8 M urea, and then purified by His-tag affinity chromatography. The eluted proteins were dialyzed against buffer containing 0.1 mM EDTA. The zymography revealed that both rSer1 and rSer2 had strong proteolytic activities.

Keywords:*Serratia liquefaciens, recombinant protein, serralysin*

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Pathology

P5

Virulence of the protein secretion system- deficient mutants of to the silkworm, *Bombyx mori*

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Pseudomonas aeruginosa, known as the human opportunistic pathogen, is a Gram-negative bacterium ubiquitously present in the environment. P. aeruginosa infects to not only the mammalian hosts, but also many organisms including the insect. In the mammalian hosts, infection mechanisms of the bacterium have been well investigated, and many important virulence factors have been identified. The infection mechanisms in the insects, however, remain unclear. In this study, we aimed to identify the important virulence factor in the insect hosts. Bacterial pathogens frequently use secreted protein to mediate interaction with their hosts. To investigate what protein is critical virulence factor for the insect, we constructed mutant strains of P. aeruginosa deficient in protein secretion systems (SSs) and assessed the virulence. P. aeruginosa has 5 type SSs including type I, II, III, V and VI. Until now, two SS-deficient mutants (type I SS, Apr pathway and type II SS, Hxr pathway) were constructed by replacing genes encoding SS with a gene cassette. The cassette contained a gentamicin resistance and a gfp gene, with both marker genes being flanked by Flp recombinase target sites. Subsequently, the cassette was excised with Flp recombinase and we obtained the markerless mutants. The assessing of virulence was carried out by inoculation with these mutants to the silkworm, Bombyx mori. The mutant strains did not show significantly decreased virulence to B. mori. This result indicated that the proteins secreted through type I SS Apr and type II SS Hxr pathway did not contribute in the virulence to B. mori.

Keywords:*Pseudomonas aeruginosa, secretion system, vurulence*

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Pathology

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Interaction of *Xenorhabdus nematophila* with *Bacillus thuringiensis* HD-73 against Bt-resistant *Plutella xylostella*

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Abstract: Xenorhabdus nematophila HB310 strain is an insect pathogen that is mutualistic with entomopathogenic nematode Steinernema carpocapsae.The oral insecticidal activities of the culture broth from X. nematophila HB310 to Bt-susceptible and Bt-resistant population of Plutella xylostella were determined by the method of leaves-tip bioassays. The results showed that the toxicity of Bacillus thuringiensis (Bt) HD-73 and Bt HD-1 to resistant population was 105.43 times and 30.70 time of susceptible population of P. xylostella larvae. However, the toxicity of X. nematophila HB310 to resistant population just only was 0.92 time of susceptible population of P.xylostella. After investigating the synergistic action between X. nematophila HB310 and Bt HD-73, we found that there was significant synergistic action of Bt HD-73 on X. nematophila HB310 against Bt-susceptible and Bt-resistant larvae of P. xylostella , however, there was not the synergistic action of Bt HD-73 on X.nematophila HB310 against Bt-susceptible and Bt-resistant larvae of P. xylostella. These results demonstrated that there was not interactive resistance between X. nematophila and Bt Hd-73 , and the insecticidal mechanism X. nematophila maybe different from that of Bt HD-73.

Keywords:*Xenorhabdus nematophila, Bacillus thuringiensis, Plutella xylostella, toxicity, synergism*

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PS5TH221

Pathology

P5

The effect of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) *Vuillemin* and *Lecanicillium muscarium* (Zimmerm.) Zare & W. Gams (Hyphomycetes: Moniliales), on mortality rate of cotton whitefly nymph

Ahmad Baghdadi[1], Kimia Kouhestani[2]

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Bemisia tabaci is a worldwide pest causing yield loss in many economically important agricultural products in green houses and open fields. Chemical control provides only short-term solutions. Moreover, the overuse of pesticides, in controlling insects, has provoked the development of resistance to insecticides. pathogenicity of Beauveria bassiana (Balsamo) Vuillemin strain DEI001 , Lecanicillium muscarium (Zimmerm.) Zare & W. Gams strain DAOM 198499 and their combinations were investigated against second instar nymphs and adults of Cotton whitefly, Bemisia tabaci Gennadius under laboratory condition. The inoculum concentrations of conidia that deposited on under sides of leaves surface were 104-108 conidia/ml and sterile distilled water with 0.04% Tween 80 (Merck) as control. Second instar nymphs were selected through stereomicroscopic observations approximately 2 weeks after adult's removal. Applications of fungi were performed with a hand held sprayer. Three or four replicates were performed per treatments. Incubation was in Binder KBWF germinator regulated to 23 ± 1° C, 95 ± 5% and 16: 8 hours (L: D) photoperiod. Assessments of dead nymphs were performed daily for 10 days after treatment. LC50s were 4.4 × 105, 1.8 × 105 and 7.9 × 105 conidia / ml with 95% confidence for B. bassiana, L. muscarium and their interaction respectively. LT50s were 4.8, 6 and 8.3 days at 108, 107 and 106 conidia/ml for B. bassiana; 3.9, 5.5 and 7.2 days for L. muscarium and 4, 5.1 and 6.7 days, for their combination. Results showed significant differences between concentrations; but no two fungi.

Keywords:*Beauveria bassiana, Lecanicillium muscarium, Bemisia tabaci, biological control, mortality rate.*

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PS6TH222

Medical Entomology

P6

COI gene information of medicinal insect *Catharsius molossus*

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Catharsius molossus is a commonly used Chinese traditional medicinal insect. After processing, the adults Catharsius molossus usually get broken to pieces and thus get hard to identify. Here we tried to use the DNA barcoding system to identify this medical insect and discriminate it from other morphologically similar segmented bug bodies which might be mixed in. We amplified a 658 bp fragment of the COI gene for 30 individuals of Catharsius molossus collected from different regions in Yunnan Province, southwest of P. R. China. Haplotypes of these individuals differed from each other by no more than five nucleotide substitutions. However, one haplotype differed from others by at least 20 nucleotide substitutions. The large COI sequence difference observed in this individual suggested that some morphologically alike species could likely be mixed in the product of the medicinal insect Catharsius molossus.

Keywords:*medicinal insect, Catharsius molossus, DNA barcoding*

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PS6TH223

Medical Entomology P6

Seasonal monitoring of dengue infection in *Aedes aegypti* and serological feature of patients with suspected dengue in 4 central provinces, Thailand

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Objectives. To determine seasonal dengue infection in Ae. aegypti mosquitoes and dengue infection in suspected patients in 4 central provinces, Thailand. Methods. Ae. aegypti mosquitoes collected twice a season and blood specimens taken from patients with suspected dengue were detected and serotyped for dengue viruses using RT-PCR. The seasonal biting behavior of Ae. aegypti females was studied by mosquito collection once a month as supportive data on dengue infection in the mosquitoes. Results. Dengue infection rates in Ae. aegypti females obtained from all the 4 provinces were highest in hot season and varied from place to place ranging from 64.4 % to 75.5%, whereas morbidity rates of DHF appeared to be highest in rainy season. The occurrence of transovarial transmission was found in local Ae. aegypti larvae and males in all provinces investigated ranging from 18.3 % to 46.9 % and from 12.0% to 46.3%, respectively. Serotyping of dengue virus in Ae. aegypti showed that DENV 3 and DENV 1 were the two most predominant serotypes, followed by DENV 2 and DENV 4. Similarly, DENV 1 and DENV 3 were the two most prevalent serotypes detected in the serum of suspected patients, followed by DENV 2 and DENV 4. The highest biting activity of Ae. aegypti females took place in hot season with the biting rate of 30 mosquitoes/person/hour. Conclusion. Data obtained from this study could be used as powerful tools for virological surveillance in Ae. aegypti populations before the occurrence of dengue outbreaks in endemic and newly dengue-introduced areas.

Keywords:*Aedes aegypti, Dengue virus, Dengue infection, Suspected patients, Biting behavior*

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Medical Entomology

P6

High throughput transcriptomic analysis to identify *Ixodes ricinus*ticks factors involved in *Bartonella henselae* transmission by ticks

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Bartonella spp. are facultative intracellular bacteria associated with several emerging diseases in humans and animals. Bartonella henselae can cause cat-scratch disease, possibly the most common zoonosis acquired from domestic animals in industrialised countries, and is becoming increasing associated with other syndromes (ocular infections, endocarditic…). We recently reported that Ixodes ricinus is a competent vector both ex vivo for B. henselae and in vivo for Bartonella birtlesii. The aim of the present project is now to investigate the parameters that govern the transmission of the bacteria by tick using the model of B. henselae transmitted by I. ricinus. For that purpose, a functional genomics approach was used to identify the genes that are differentially expressed in ticks in response to infection with B. henselae. Larvae and succeeding nymph ticks were artificially engorged via artificial membrane feeding technique on non-infected or B. henselae-infected blood. Resulting females (87 females for the infected group and 69 for the non-infected one) were then pre-engorged for 4 days in order to stimulate the development of salivary glands. Salivary glands were then dissected and frozen until total RNA extraction. The salivary glands transcriptomes of B. henselae infected and non-infected I. ricinus females were sequenced using high throughput sequencing technologies, and the comparison between the two conditions as well as blast researches were performed. In the present work we report the first results obtained from this analysis. In the future, the role of the differentially expressed genes in the pathogen transmission and development process will be investigated.

Keywords:*ticks, Bartonella, high throughput sequencing, transmission*

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VectorMap: Leveraging spatial data on agents of febrile vector-borne infections in support of disease risk assessments

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VectorMap (www.vectormap.org) is a web resource for mapping occurrence data and distribution models of agents, primarily arthropods (mosquitoes, ticks, sand flies), involved in febrile vector borne-infections (FVBI). Collection data points, and climate and remote sensing data, are used with ecological niche and other distribution models, to produce predictions of the vector and disease distribution. Disease distribution layers, in an online GIS setting, allow military entomologists and DoD Force Health Protection workers to assess risks worldwide. VectorMap provides critical information on geography/demography, past vector collection data, the possible extent of vector species, and the spatial relationship of vectors to diseases and animal hosts/reservoirs. Mal-area Calculator (MAC) quantifies the overlap of humans, vectors, and disease, in order to estimate vector hazard and FVBI risk for any area of interest. VectorSurv application helps to understand when vector population densities are above average by providing information-rich graphs of species seasonality and abundance. Thus, VectorMap delivers the following: the preservation of valuable data, the display of data in a way that assists infection risk analyses, the promotion of data sharing within AFHSC-GEIS network, and the building of standards and skills.

Keywords: *VectorMap, vector-borne infections, disease risk*

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Medical Entomology

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Feeding efficiency of Odonate species and *Poecilia reticulata* against 3 mosquitoes species in Malaysia

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The use of guppies (Poecilia reticulata) as biocontrol for mosquitoes are well documented but in contrast the use of dragonfly nymphs have been little studied. The efficiency of odonate species, Neurothemis fluctuans, Orthetrum sabina, Orthetrum chrysis and Poecilia reticulate as biocontrol against three common mosquito species in Malaysia, Aedes albopictus, Aedes aegypti and Culex quinquefasciatus larvae were tested under laboratory conditions. The daily feeding rates varied among predators and mosquito species. The mean number of IV instar mosquito larvae consumed by the predators was different between the mosquito species. Aedes aegypti were the most preferred prey for Orthetrum sabina followed by Poecilia reticulata and Neurothemis fluctuans, in the order of 73, 70 and 66 respectively. However, Orthetrum chrysis consumed more Culex quinquefasciatus as a choice prey. Behavior of both mosquito larvae species and predators showed direct influence on the the predatory activities. The feeding rates varied between day time and night time. All predators exhibited diurnal activities being day time stalkers and actively consuming more mosquito larvae during the day compared to night time. The efficiency of predators depended on several factors which are explained in the paper, mainly: escape behavior of prey, search ability and body sizes of predators This study thus lend support to the potential use of odonate species as biocontrol in managing the mosquito species population.

Keywords:*biocontrol, predator, prey, behavior, mosquito larvae*

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Role of the conserved BBA68-BBA73 operon in the life cycle of Lyme spirochete, *Borrelia bugdorferi*

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Lyme disease is a global, emerging tick-borne disease caused by the bacterial pathogens of Borrelia burgdorferi s.l. The Lyme spirochete is widely distributed, and hundreds of cases were reported in northeast China each year. In its life cycle, Lyme spirochete dramatically alters the protein profiles when it is transmitted between ticks and mammals. Now, it is proposed that a central signaling pathway, the Rrp2-RpoS pathway, modulates expression of many genes that are vital to mammalian infection. Accumulated data also showed that the BBA68-BBA73 operon partially regulated by Rrp2-RpoS pathway might play a role in this infection process. To elucidate the role of BBA68-BBA73 protein in the enzootic cycle of B.burgdorferi, mutation of bba68 ,bba69 ,bba70 ,bba71and bba73 each gene was generated in an infectious strain of B.burgdorferi by homologous recombination. Groups of C3H/HeJ mice were inoculated intradermally with 106 spirochetes of either wide-type or isogenic mutant spirochete. At 3 weeks after inoculation, mouse tissues (ear, joint, bladder and heart) were collected and cultured in BSK-H medium with 1×Borrelia antibiotic mixture. Our results showed that BBA68, BBA69, BBA71 but not BBA70 and BBA73 are essential for mammalian infection by needle inoculation. The biological role of these proteins in the enzootic cycle of B.burgdorferi employed by tick-mouse model is under investigation.

Keywords:*Lyme disease, BBA68-BBA73*

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The first molecular identification of *Borrelia* spp. in *Rhipicephalus sanguineus* (Acari: Ixodidae) from domestic dogs in Taiwan

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Lyme disease is an emerging tick-borne spirochaetal infection that can lead to multisystem human illness. It is caused by Borrelia burgdorferi sensu lato. Human disease is associated with at least three Borrelia species, B. burgdorferi sensu stricto, B. afzelii, and B. garinii. Recently, the first laboratory-diagnosed human case of Lyme disease caused by B. garinii has been reported in Taipei City in northern Taiwan and B. burgdorferi spirochaetes have been isolated from rodents in the Kimmen County, which is located offshore from the mainland of China. However, the vector species responsible for the transmission of human Lyme disease is still unknown. In this study, brown dog ticks (Rhipicephalus sanguineus) were collected from domestic dogs in the animal hospitals in New Taipei City. The 16S rRNA gene of Borrelia spp. was the target of molecular identification by polymerase chain reaction. Whilst the overall infection rate of Borrelia spp. was 11.25% (16/128), Borrelia spp. were detected in 5 (28%), 2 (11%), 6 (33%) 1 (6%) and 2 (11%) of the samples collected in August, September, October, November, and December in 2011, respectively. Sequences shared 99% homology with both B. burgdorferi and B. garinii. To our knowledge, this is the first report of the Borrelia infection among R. sanguineus in Taiwan. Since parasitism by R. sanguineus amongst humans has become an emerging public health problem, the study of the interactions amongst the Borrelia spp. and R. sanguineus can provide the critical information required for the control and prevention of Lyme disease amongst humans.

Keywords:*brown dog tick, Rhipicephalus sanguineus, Borrelia garinii, Taiwan*

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Medical Entomology

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Periungual tungiasis and *Rickettsia felis* in cat fleas in the Democratic Republic of Sao Tome and Principe, West Africa

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A healthy 26-year-old Taiwanese man working in the Democratic Republic of Sao Tome and Principe (DRSTP) in West Africa, presented with a brownish lesion on the medial edge of his right third toe. Examination found multiple eggs of sand flea Tunga penetrans after applying lateral pressure. The lesion contained hindquarters of the dead female sand fleas, which deposit hundreds of eggs in the skin tissues. The infestation usually occurs over unprotected feet. After the excision of the embedded jigger flea from the brownish lesion, he recovered completely. Rickettsia felis is the etiologic agent of flea-borne spotted fever and has been discovered in fleas throughout the world. There have been a few human cases of R. felis infection or data of vector surveillance reported in Africa, including Algeria, Democratic Republic of Congo, Ethiopia, Gabon, Ivory Coast and Tunisia in the last decade. In this study, we describe the first molecular detection of Rickettsia felis in cat fleas (Ctenocephalides felis) in the DRSTP. A total number of 1,092 fleas were collected from domestic dogs in Cantagalo and Agua Grande Province in August 2011. Morphological identification shows these fleas were all Ct. felis. Natural infections of R. felis in cat fleas were isolated and confirmed using polymerase chain reaction. The infection rate in individual flea was found to be 25.11% (57/227). Partial sequences of the citrate synthase, outer membrane protein A, and specific plasmid pRF genes obtained from the samples are 100% identical to those of R. felis URRWXCal2.

Keywords:*Tunga penetrans, Ctenocephalides felis, Rickettsia felis, Democratic Republic of Sao Tome and Principe*

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New approaches to physical mapping of mosquito genomes

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Mosquitoes are vectors of numerous deadly human diseases. To facilitate the development of new strategies for vector control, the genomes of the three most dangerous species of mosquitoes had been sequenced in the last decade. Among these three species, the vector of dengue and yellow fever viruses, Aedes aegypti, has the largest genome with 1,310 Mb, which is hard to assemble and map to chromosomes. Here, we report the first band-based chromosomal map of the Ae. aegypti genome. Idiograms for the mitotic chromosomes of Ae. aegypti were developed. Instead of previously used cell lines, which usually accumulate chromosomal rearrangements, our method utilized chromosomes from a live mosquito. Based on fluorescent in situ hybridization (FISH), a total of 500 BAC clones was assigned to the specific band onto idiograms. From all BAC clones 106 were carrying previously mapped major genetic markers. These BAC clones were additionally ordered within each band by multicolor FISH because of the importance to link their genomic locations to the genetic locations of quantitative trait loci (QTL) related to pathogen transmission. The current study placed ~50% of the Ae. aegypti genome to precise chromosomal positions and also combined cytogenetic, genetic and genome maps into one integrated physical map (iMap) of the yellow fever mosquito. Further application of this map will enhance the quality of the current genome assembly of Ae. aegypti and also will help to find the genomic locations of QTL that might be important targets for developing advanced genome-based strategies for vector/disease control.

Keywords:*mosquito genome mapping*

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Insecticide resistance in bed bugs in Thailand and laboratory evaluation of insecticides for the control of *Cimex hemipterus* and *Cimex lectularius* (Hemiptera: Cimicidae)

Apiwat Tawatsin[1], Usavadee THAVARA (1)[2], Jakkrawarn Chompoosri[3], Yutthana Phusup[4], Nisarat Jonjang[5], Chayada Khumsawads[6], Payu BHAKDEENUAN (1)[7], Pathom Sawanpanyalert[8], Preecha Asavadachanukorn[9], Mir S. Mulla[10], Padet Siriyasatien[11], Mustapha Debboun[12]

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Bed bugs are found in many countries around the world and in some regions they are resistant to numerous insecticides. This study surveyed bed bugs in Thailand and determined their resistance to insecticides. The surveys were carried out in six provinces which attract large numbers of foreign tourists: Bangkok, Chonburi, Chiang Mai, Ubon Ratchathani, Phuket and Krabi. Bed bugs were collected from hotels and colonized in the laboratory to evaluate their resistance to insecticides. Cimex hemipterus (F.) was found in some hotels in Bangkok, Chonburi, Phuket and Krabi; whereas, C. lectularius L. was found only in hotels in Chiang Mai. No bed bugs were found in Ubon Ratchathani. The colonized bed bugs showed resistance to groups of insecticides, including organochlorines (DDT, dieldrin), carbamates (bendiocarb, propoxur), organophosphates (malathion, fenitrothion) and pyrethroids (cyfluthrin, deltamethrin, permethrin, lambda-cyhalothrin, etofenprox) in tests using World Health Organization (WHO) insecticide-impregnated papers. The new insecticides imidacloprid (neonicotinoid group), chlorfenapyr (pyrrole group) and fipronil (phenylpyrazole group) were effective against the bed bugs; however, organophosphate (diazinon), carbamates (fenobucarb, propoxur) and pyrethroids (bifenthrin, cypermethrin, esfenvalerate, etofenprox) were ineffective. Aerosols containing various pyrethroid insecticides with 2-4 different active ingredients were effective against the bed bugs. The results obtained from this study suggested that both species of bed bugs in Thailand have developed marked resistance to various groups of insecticides, especially those in the pyrethroid group which are the most common insecticides used for pest control. Therefore, an integrated pest management should be implemented for managing bed bugs in Thailand.

Keywords:*Bed bugs, Control, Insecticide resistance*

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Morphological and molecular phylogenetic studies on a novel anopheline species collected in Kushiro wetland, northern Japan

Nozomi Imanishi[1], Kenji Takai[2], Kyeong Soon Kim[3], Yoshio Tsuda[4], Mutsuo Kobayashi[5], Kyo Itoyama[6], Kyoko Sawabe[7]

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Since 2004, we have been aware that there exists a population of anopheline mosquitoes in Kushiro wetland, northern Japan, that have a unique DNA sequence different from any of Anopheles sinensis, An. engarensis, An. lesteri and An. sineroides. Based on phylogenetic analysis of internal transcribed spacer 2 (ITS2) sequences of ribosomal DNA, a novel anopheline species was identified to be An. belenrae described from Korea in 2005. Recently we collected An. belenrae mosquitoes in surrounding areas of Kushiro wetland, suggesting a wide distribution of the species in eastern Hokkaido. Phylogeny as well as morphological studies between An. belenrae from Kushiro and other members of Anopheles species inhabiting Japan have never been made. Since An. belenrae inhabiting Kushiro usually has fringe pale spot (Fp) on its wings and is quite similar to An. sinensis and An. engarensis, we conducted more thorough examination to see morphological and phylogenetic differences among An. belenrae and two sibling species, An. sinensis and An. engarensis. Morphological characteristics in adult, pupa and larva were examined and compared. All morphological characteristics examined in this study showed some overlaps among the three species and no definite morphological identification keys were found. Partial ITS2 sequences (491bp) of An. belenrae diverged 14% from both of An. sinensis and An. engarensis. We concluded that An. belenrae inhabiting Kushiro wetland can be well distinguished from An. sinensis and An. engarensis at the DNA level by ITS2 sequences, however no definite morphological identification keys were available at present.

Keywords:*Anopheles belenrae, Japan, morphology, ITS2*

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Silkworms in National Bio-Resource Project (NBRP) of Japan

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National Bio-Resource Project (NBRP) started in 2002 with support by the Ministry of Education, Culture, Sports, Science and Technology Japan. Two research spans (five years each) were already finished and the third season will be started at April, 2012. 28 species including silkworm, medaka (killifish), morning glory, Japanese monkey, arabidopsis and rice are opened for worldwide use. Purpose of NBRP is Collection, Preservation and Distribution of bio-resources that are basic materials for life science researches. Silkworms of NBRP are available by contact to http://www.nbrp.jp/report/reportProject.jsp?project=silkworm. 1.Domesticated silkworm, Mutant strains (500), Improvement strains for artificial diet (200), Transgenic strains (100), 2.Wild silkworm, Antheraea yamamai (5), Antheraea pernyi, Samia cynthia (1), 3. Genomic clones (220,000)(DNA / cDNA) from 50 libraries. When you have some interests for mutants, you can order it every time. Generally, exchange or distribution of eggs is limited in spring season. However, eggs are available during all months by us. For the purpose, we prepare two kinds of eggs in June. One is available for July to early November by the acid-treatment. Another one is available from late November to next April by the artificial hibernation. November is a little bit weak for distribution, but most months are okay to distribute the eggs by this project. For order of the eggs, maternal transfer agreement (MTA) is needed for protection of the property rights of breeder or developer. We try to get foreign country's stocks. We should discuss for the construction of the international organization for information exchanges and network.

Keywords:*Bombyx mori, Strain, Genome resources, Transgenic silkworm, Wild silkworm*

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Medical Entomology

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Odor-based contagious transmission of pathogen by *Drosophila melanogaster*

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The housefly and flies are considered to be mechanical vectors of many kinds of pathogens, whereas mosquito serves as the biological vector for pathogens. Mechanical vectors simply convey pathogens and are not essential for their development and life cycle. In order to uncover the molecular mechanisms of transmission by fly, we established a model system for transmission using Drosophila melanogaster. GFP-labeled Escherichia coli located on the center of an agar-plate was freely ingested by Drosophila. Substances excreted in the feces are observed as small spot with fluorescence on the surface of agar, showing that flies directly feed E. coli and disseminate them by excretion. Flies without antennae that contain a large set of olfactory receptors or deficient for Or83b, which encodes a broadly expressed odorant receptor, showed impaired dissemination of bacteria. While wild-type flies showed behavioral responses to attractive odors released from growing E. coli, Or83b mutants failed to respond to these odors. Volatile compounds emitted from culture supernatant of E. coli were trapped and identified by gas chromatography-mass spectrometry (GC-MS). The predominant compound produced by E. coli was indole along with lesser amounts of alcohols. We also showed that LUSH, Drosophila orthologue of indole-binding protein, is required for transmission of E. coli as excreted droplets. Given that Drosophila LUSH is also known as a component to activate pheromone-sensitive neurons, we therefore suggest that the pheromone-mediating system may also promote feeding behavior in the presence of indole from pathogens, contributing to the transmission of infectious diseases such as food poisoning.

Keywords:*fly drosophila transmission pathogen odor indole*

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Distribution of biting midges *Leptoconops spinosifrons* (Carter) (Diptera: Ceratopogonidae) in Thailand and field efficacy of herbal repellent against *L. spinosifrons*

Usavadee Thavara[1], Apiwat Tawatsin[2], Jakkrawarn Chompoosri[3], Somchai Saengkitporn[4], Preecha Asavadachanukorn[5], Padet Siriyasatien[6], Arunrat Thepparat[7], Mir S. Mulla[8]

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Biting midges Leptoconops spinosifrons (Carter) are annoying pests found on the shorelines and sandy beach areas. In Thailand, L. spinosifrons has been found on various beaches in 9 provinces (Trat, Chantaburi, Rayong, Chonburi, Prachuab Kirikhan, Chumporn, Surat Thani, Nakhon Si Thammarat, Songkhla) around the Gulf of Thailand and 6 provinces (Ranong, Phang Nga, Krabi, Trang, Satun, Phuket) along the Andaman Sea, including some islands located in both seas. The biting midge has two peaks of biting activities (0700-0900 hours and 1600-1800 hours). It feeds on tourists and resident visiting the beaches and resorts. These insects deter human recreation and diminish Thailand tourism. Since the control of biting midges along beaches by larvicides or adulticides is hardly possible, attempts have been made then to prevent its nuisance by using repellent application. An herbal repellent, containing essential oils from Eucalyptus citriodora Hook. and Litsea cubeba (Lour.) Pers., was evaluated for repellency against L. spinosifrons in comparison with N,N-diethyl-3-methylbenzamide (deet). Both repellents were prepared as 20% in ethanol and each one was applied on the clothes (long sleeve T-shirt and pants) at the dosage of 0.5 mg a.i./cm2. Assessments were made using landing rates on the treated clothes as compared with controls (untreated clothes). Both repellents showed relatively high repellency against L. spinosifrons; however, the herbal repellent provided higher degree of repellency (98.3-99.7%) than did deet (82.7-98.5%). This study demonstrates a high potential for use of the herbal repellent to protect from biting of L. spinosifrons.

Keywords:*Biting midge, Leptoconops, Thailand, Repellent*

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Medical Entomology P6

Comparative detection of chikungunya and dengue virus in *Aedes albopictus* Skuse collected from Surat Thani province, Thailand by multiplex real-time RT-PCR and conventional RT-PCR

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This study evaluated two assay methods, multiplex real-time RT-PCR and conventional RT-PCR for detection of chikungunya virus (CHIKV) and dengue virus (DENV) in Aedes albopictus Skuse collected from Surat Thani province, Thailand. The mosquitoes were collected from endemic areas of the province and extracted for viral RNA. The same amount of viral RNA was further processed to detect CHIKV and DENV using the two methods. The positive pools of mosquitoes were determined for chikungunya and each serotype of dengue infection which was used for evaluation of the two methods. One hundred of mosquito pools were assayed for both viruses by both methods. Multiplex real-time RT-PCR could detect CHIKV, DEN-2, and co-infection of DEN-2 and DEN-4 in 24 pools, 9 pools and 1 pool with the infection rate of 24%, 9% and 1%, respectively. In contrast, the conventional RT-PCR found 6 pools positive for CHIKV, with an infection rate of 6% and only DEN-2 was detected in 2 pools with an infection rate of 2%. The chikungunya and dengue positive pools detected by conventional RT-PCR were consistent with those determined by real-time RT-PCR. Real-time RT-PCR assay is more sensitive and reliable than conventional RT-PCR for detection of both viruses in field-caught Ae. albopictus mosquitoes. Since real-time RT-PCR assay provides advantages over conventional RT-PCR, including simple process, easy standardization, rapidity, lower contamination rate and higher sensitivity. It might replace conventional RT-PCR as a powerful tool for rapid detection of viruses in vector mosquitoes and other assayed samples.

Keywords:*Chikungunya virus, Dengue virus, Aedes albopictus, RT-PCR*

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Medical Entomology P6

Molecular identification of forensically important fresh flies (Diptera: Sarcophigidae) in Thailand by using the second internal transcribed spacer (ITS2) gene

Payu Bhakdeenuan[1], Kanok Preativatanyou [2], Sunchai Payungporn[3], Usavadee Thavara[4], Apiwat Tawatsin[5], Kom Sukontason[6], Kabkaew L. Sukontason[7], Wej Choochote[8], Padet Siriyasatien[9]

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In order to determine post mortem interval (PMI) using flies collected from corpses more precisely, accurately identification of flies is essential. Morphology identification of fly especially in the same genus is difficult and required highly expert personals. In this study we evaluate the using of molecular techniques for identification forensically important sarcophigid flies in Thailand using ITS2 gene. A total of 26 specimens of four sarcophigid fly species (Sarcophaga dux, S. ruficornis, S. peregrina and S. scopariiformis) collected from various regions of Thailand were used. When compared sequences with database, the percentage identities range from 99 to 100. Intraspecific variation of each species was 0-0.3%, while the maximum interspecific variation was 19.7%. PCR-RFLP was able to differentiate these four sarcophigid species. The results of this study would be valuable for forensic personnel in order to identify species of forensically importance flies collected from the crime scenes more accuracy and therefore accurate estimate of the PMI.

Keywords:*Sarcophigid fly, Internal Transcribed Spacer 2, Molecular identification, PCR-RFLP, Thailand*

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Medical Entomology

P6

The bacterial and fungal florai associated with digestive system of *Blattella germanica* L. collected in spring and autumn from dormitories and dwellings in Mashhad, Iran

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The present study aimed to study the bacterial and fungal flora associated with the digestive system of Blattella germanica L. collected in spring and autumn from dwellings and dormitories in Mashhad, Iran. After dissection of digestive system under sterile condition, the extracts were incubated in EMB and Blood Agar medium. The result showed that in autumn, the most prevalent bacteria in dwellings and dormitories were Klebsiella oxytoca (80%) and Entrobacter aerogenes (96%), respectively. The flora of bacteria associated with dwellings consisted of Enterococcus spp., Staphylococcus spp., Klebsiella spp., Entrobacter aerogenes and Micrococcus spp. All these species except the latter were found in dormitories. Moreover, two species including Proteus vulgaris and P. mirabilis were found in dormitories. The results indicated that 11 out of 50 cockroaches (22%) from houses and 12 (24%) from dormitories were contaminated with fungi. In spring, the most prevalent bacterium in dwellings and dormitories was Enterococcus spp. with the frequencies of 43% and 68%, respectively. The flora of bacteria associated with dwellings consisted of Enterococcus spp., Staphylococcus spp., Klebsiella spp., Entrobacter aerogenes, Serratia marsesens, Proteus spp., Escherichia coli and Tettragen spp. All these species except the latter were found in dormitories. The results indicated that 10 out of 50 cockroaches (20%) from houses and 1 cockroach (2%) from dormitories were contaminated with fungi. The results suggested that for reducing the risk of transmission of various disease agents, suitable control measures should be applied to manage the population of cockroaches.

Keywords:*Spring, autumn, bacteria, fungi, German cockroach, dormitories, dwellings, Mashhad*

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Chromosomal evolution in *Anopheles* mosquitoes

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Anopheles gambiae and An. funestus are primary malaria vectors in Africa, and An. stephensi plays an important role in malaria transmission in Asia. An. gambiae and An. funestus diverged from each other at least 36 million years ago, and An. stephensi is closer related to An. gambiae than to An. funestus. Therefore, these distantly related species are an excellent system for studying the dynamics of chromosomal evolution because inversions are highly nonuniformly distributed among five chromosomal arms. To determine the rates of inversion fixation and better understand the evolutionary forces that govern the unequal distribution of rearrangements among chromosomes, a physical map for A. stephensi, was created and compared with the maps of the major African malaria vectors A. gambiae and A. funestus. The study of paracentric inversions between lineages in Anopheles mosquitoes demonstrated that, despite the paucity of inversion polymorphisms on the X chromosome, this chromosome has the fastest rate of inversion fixations and highest density of transposable elements, simple DNA repeats, and GC content. The highly polymorphic and rapidly evolving autosomal 2R arm has overrepresentation of genes involved in cellular response to stress supporting the role of natural selection in maintaining adaptive polymorphic inversions. In addition, the 2R arm has the highest density of regions involved in segmental duplications that clustered in the breakpoint-rich zone of the arm. In contrast, the slower evolving 2L, 3R, and 3L, arms are enriched with matrix attachment regions that potentially contribute to chromosome stability in the cell nucleus.

Keywords:*Anopheles stephensi, chromosomal rearrangements, inversion fixation, evolution rate*

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Efficacy of lethal ovitraps against *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Diptera: Culicidae)

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Ovitraps are artificial breeding containers attracting gravid female mosquitoes to lay eggs and they have been used for surveillance of dengue haemorrhagic fever (DHF) and chikungunya vectors for years; however, these ovitraps could be also modified as tools for integrated vector control. To obtain an effective lethal ovitrap, evaluations of substrate preferences and response of Aedes aegypti (L.) and Ae. albopictus Skuse to ovitraps treated with larvicides were then carried out under laboratory and field conditions. In laboratory, the brown paper substrate showed higher attractiveness for oviposition of both mosquito species than the other candidate substrates (filter paper, sponge sheet and hardboard paddle). Regarding larvicidal comparison, the mean number of eggs laid in ovitraps containing temephos and Bacillus thuringiensis israelensis (Bti) were significantly higher than those laid in ovitraps containing novaluron and untreated ovitraps. No ovicidal effect of the larvicides used in ovitraps was observed since hatching of eggs occurred; however, all larvae died eventually. In field experiments, evaluations of lethal ovitraps having the brown paper as egg-laying substrate treated with temephos compared with the untreated ovitraps were carried out in rubber plantation habitats in Trang province, southern Thailand. There was no significant difference between the mean number of eggs laid in the lethal ovitraps and those laid in the untreated ovitraps. This clearly indicated that no repellent effect was observed in the lethal ovitraps. The lethal ovitrap obtained from this study could possibly be used as an effective, economical and simple mean of DHF and chikungunya vector control.

Keywords:*Ovitrap, Aedes aegypti, Aedes albopictus, DHF, Chikungunya*

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Insecticidal efficacy of plant extracts against common bed bug *Cimex lectularius* L. (Hemiptera: Cimicidae)

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Bed bugs become serious urban pest in many countries around the world because they are resistant to numerous insecticides commonly used for pest control. Attempts were then made to search for effective and safe materials to control the insecticide-resistant bed bugs. In Thailand, there are numerous plants containing insecticidal effects against various kinds of insects. Extracts from 15 plant species were tested for insecticidal efficacy against Cimex lectularius L. under laboratory conditions. It was found that the fixed oil extracted from Annona squamosa L. and the essential oils extracted from Alpinia galanga (Linn.) Swartz. and Syzygium aromaticum (L.) Merr. & Perry. provided satisfactory insecticidal efficacy against the bed bugs with mortality rate of about 60-80%. These three extracts were then further formulated as oil in water (O/W) emulsion spray and aerosol and tested against bed bugs. Interestingly, the formulated spray and aerosol showed excellent insecticidal efficacy against the bed bugs. The results obtained from this study demonstrated that the plant extracts containing insecticidal effects could be formulated to enhance their efficacies and they could possibly replace synthetic insecticides commonly used for bed bug control.

Keywords:*Plant extracts, Bed bugs, Control*

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Metarhizium anisopliae as a potential biopesticide for the brown-banded cockroach, *Supella longipalpa* (Blattaria: Blattellidae)

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The brown-banded cockroach, Supella longipalpa (F.), is one of the common household pests, while Metarhizium anisopliae (Metschn.) Sorok is an entomopathogenic fungus, safe to human, mammals, and environment. The pathogenicity of M. anisopliae against the brown-banded cockroach was evaluated using three common household pest management techniques i.e., spraying, bait, and pheromone trap. The mortality and LT50 value were 78.3% and 9.8 days, respectively, when S. longipalpa was sprayed directly with a M. anisopliae suspension at 1.6×10^8 conidia/ml. The suspension was sprayed on the surface of different materials commonly used for interior decoration in which those with smoother surface caused higher mortality; for example, plastic > tile > glass > stainless sheet steel > wood, when applied at 1.6×10^9 conidia/ml. The bait was prepared by combining the preferred food of the cockroach with the M. anisopliae conidia. Its fecal extracts extracted with methanol or methylene chloride were able to enhance fungal infection and the bait performance. The trap containing methanol fecal extracts increased 22.0% of the capture rate compared with the control group. Our assays using a ratio of 1:10 (exposed : unexposed to conidia) resulted in 30.0% mortality in healthy colonies, indicating that M. anisopliae can be transmitted horizontally within S. longipalpa populations. Therefore, M. anisopliae could be a potential biopesticide for managing this cockroach when applied alone or in combination with food baits or with the bait stations containing fecal extracts.

Keywords:*biopesticide, brown-banded cockroach, entomopathogenic fungus, Metarhizium anisopliae, Supella longipalpa*

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Breeding sites of main Bluetongue virus vectors in Belgian cowshed

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Bluetongue (BT) is an emerging vectorborne disease of ruminants that was reported in August 2006 in northern Europe. Since 2007, BT virus (BTV) serotype 8 continued its spread across Europe and caused considerable economic losses. This observation indicates possible overwintering of the vector from year to year. The biological vectors of BTV are biting midges of the genus Culicoides. Breeding sites of bluetongue vector species have been found near farms (e.g. silage residues) and in neighboring meadows (e.g. cattle dung) but never inside sheds. We conducted a study in five cattle farms in Belgium during February?October 2008. Three samplings were performed and each soil sample collected inside cowsheds was incubated to enable adult midges to emerge. Among 15 soil biotopes sampled, only one showed the emergence of adult Culicoides biting midges: dried dung adhering to walls inside animal enclosures and resulting to the partial removal of used animal litter. It was a breeding site for the C. obsoletus/scoticus complex. Physico-chemical characteristics showed that midges of this complex are more prevalent in soil samples with a high carbon:nitrogen (C:N) index. So Culicoides biting midges are able to complete their life cycle in animal enclosures. We identified a breeding site for the primary BTV vector in a cowshed in northern Europe. These observations could explain the persistence of BTV from year to year despite fairly harsh winters. Hygienic measures on farms could reduce biting midges populations and so improve efficacy of vaccination campaigns against BT in Europe.

Keywords:*Culicoides, Bluetongue, Vector, Breeding site, Cowshed, C. obsoletus, scoticus complex, C:N index, Hy*

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Laboratory evaluation of efficacy of *Bacillus thuringiensis* Crude toxin (Bt.Ct) and Bt.Ct synthesized silver nanoparticles (Bt.Ct-AgNps) against human vector mosquitoes

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A comparative study was carried out under laboratory condition using Bt.Ct and Bt.Ct-AgNps against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi. Bt.Ct synthesized AgNps was characterized and conformed using UV-vis spectra, SEM and EDX. The bioassay study using Bt.Ct revaled the present cumulative mortality of 97.8%, 90% and 93.33% with respect to A. aegypti, Cx. Quinquefasciatus and An. stephensi thus, tested bacterial strain would be a potent larvicide against Human vector mosquitoes and can also be recommended for the field evaluation.

Keywords:*Bacillus thuringiensis, Silver Nanoparticles, Aedes aegypti, Culex quinquefasciatus, Anopheles steph*

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A long-term pattern of prevalence of dragged ticks captured in the Korean Peninsula from 2001 to 2010

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Many infectious diseases of humans have been increasingly emerged as zoonoses transmitted by vectors. Vector-borne diseases are a major contributor to the global health burden in the world. Among the vectors, tick-borne diseases have risen dramatically over the last two decades. Here, a long-term prevalence of Korean tick-borne diseases was shown together with information of Korean tick species. Samplings of ticks were carried out during the last ten years (2001-2010) and ten infectious agents were screened in this study. A total of 16,375 ticks were collected from the wild habitats by dragging. Based on the results, seven tick species belonging to three genera (Haemaphysalis, Ixodes and Amblyomma) were found from seven provinces during the study period. Except unidentified ticks, H. longicornis (n=8828; 53.91%) was the most frequently sampled tick, followed by H. flava (n=5593; 34.16%), I. nipponensis (n=753; 4.49%), I. turdus (n=21; 0.13%), A. testudinarium (n=20; 0.12%), H. Japonica (n=12; 0.07%) and I. persulcatus (n=8; 0.05%). Larvae (n=7858; 47.99%) and nymphs (n=7597; 46.39%) of Haemaphysalis spp. and Ixodes spp. were sampled most frequently but only nineteen nymphs and one male adult of A. testudinarium were sampled by dragging. In addition, from 2001 to 2009, various infectious agents were detected with a unique Minimum Field Infection Rate (MFIR). Among them, Rickettsia spp. showed the highest MFIR (6.29%), followed by Bartonella spp. (1.67%), Ehrlichia chaffeensis (1.42%), Borrelia burgdorferi (1.33%) and Anaplasma platys (1.26%). This long-term monitoring of dragged ticks would be helpful to understanding the distribution of ticks, abundance and infection.

Keywords:*dragged tick, Korean peninsula, Haemaphysalis, Ixodes, Amblyomma, MFIR, prevalence*

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P6

Repellent efficacy and safety evaluation of IR3535 derivative against *Aedes albopictus, Culex pipiens pallens* and *Aedes togoi*

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The IR3535 derivative (LJH158), in which the ethyl ester of IR3535 was converted to the methyl ester, was synthesized and studied as the new mosquito repellent ingredient. The repellent efficacy of LJH158 was compared with that of DEET against Aedes albopictus, Culex pipiens pallens and Aedes togoi. Also, the aromatic repellent tests were conducted with the mixtures of repellents and the essential oils of cinnamon which were obtained by supercritical extraction. In addition, the safety issues of LJH158 were monitored through single oral dose safety study, eye irritation test, and skin irritation test. The results of repellent efficacy in both biting and aromatic tests and safety tests suggest that LJH158 has high potential to be used as a new repellent ingredient.

Keywords:*mosquito, repellent, IR3535, LJH158, Cinnamon, supercritical extraction*

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Influence of antimicrobial peptides over-expressed in damaged gut cells of *Drosophila melanogaster*

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To investigate the cause of Inflammatory bowel disease (IBD) by using Drosophila melanogaster, we created a D. melanogaster intestinal damage model by orally feeding the flies with various IBD inducers. After feeding the flies with IBD inducers of LD50, we found that in the damaged gut cells, the number of enteroendocrine cells secreting antimicrobial peptides (AMPs) had increased and the expression level of the AMPs, and upstream signalling of the AMPs (Toll, Pelle, Dorsal and Relish) had up-regulated. We also confirmed that apoptosis and intestinal stem cells (ISCs) had markedly increased in the damaged gut cells. On the other hand, results obtained by using RNA interference (RNAi) lines for the knockdown of various AMP genes showed that the survival rate had increased and apoptosis had decreased in the RNAi lines as compared to wild-type flies. Anti-Toll, anti-Dorsal, and anti-Pelle antibody feeding flies survived longer than normal flies after feeding with DSS. We deduce from the above results that when IBD inducers entered the fly gut, various AMPs were up-regulated for self-defense. Over-expressed AMPs not only function in intestinal protection by destroying harmful microorganisms, but also function in intestinal destruction by attacking their own cellular components. Hence, we suggest that AMPs that are over-expressed by IBD inducer could be a possible cause of IBD.

Keywords:*Amtimicrobial peptide, Drosophila melanogaster, Inflammatory Bowel Disease (IBD)*

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Allergenicity of recombinat alpha-amylase from German cockroach

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Cockroach produces potent allergens. Especially, feces are known to be rich in allergens. By the analysis of German cockroach fecal extract, α-amylase was found to be allergenic. German cockroach α-amylase shared the highest identity with pig α-amylase (56.5%), followed by mite group 4 allergens (Blo t 4, 50.6%; Der p 4, 50.0%; Eur m 4, 47.6%). In this sduty, recombinant α-amylase from German cockroach was expressed and its allergenicity was examined by ELISA. Specific IgE against recombinant amylase was detected from 40 % (12/30) of serum samples from German cockroach-sensitized subjects. Amylase is found to be one of the important allergen in cockroach feces. It is hoped that recombinant amylase will be useful for further studies and clinical applications.

Keywords:*Amylase, Allergen, Cockroach*

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P6

Carbamate insecticides bioassays and resistance monitoring in field strains of adult German cockroach, *Blattella germanica* (L.), from southern Iran

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The German cockroach, Blattella germanica L. (Dictyoptera: Blattellidae), has become resistant to many insecticides due to control failures. This study was designed to detect and monitor carbamate resistance in field strains of German cockroach by using lethal dose bioassay methods. Two wild German cockroach strains (dormitory 'D' and hospital 'H') were collected. Adult males were subjected to the jar exposure procedure. A range of concentrations starting with WHO standard concentration of carbamate insecticides (carbaryl, bendiocarb, propoxur) were used in a jar bioassay format. For each, four to seven different concentrations leading to >0% and <100% mortality were used. Ten cockroaches were placed in each jar and the exposure time of contact was held constant for 30 minutes. Mortality data and slopes from the replicates were assessed using probit analysis. Although both strains showed lethality values above those of the WHO standards, the 'H' strain was more resistant to all of the three carbamate insecticides than the 'D' strain. The order of resistance for 'D' strain was carbaryl> propoxur > bendiocarb, while the order of resistance for 'H' strain was carbaryl> bendiocarb > propoxur. The ratio of LD50 in 'H' strain to that of 'D' strain for carbaryl was about twice that of the other two insecticides. It is concluded that heavy reliance on carbaryl in both 'D' and 'H' settings has led to resistance and control failures. A set of basic rotations with mosaics of different classes of insecticides may be used in future.

Keywords:*Blattella germanica, Carbamate, Insecticide, Cockroach, Resistance, Iran*

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P6

Scabies disease prevalence and social factors affecting referrals to health centers in Hamadan and Kermanshah provinces during the years 2006 -2011

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Scabies, a parasitic skin infestation by the burrowing "itch" mite Sarcoptes scabiei, causes significant health problems for children and adults worldwide. Due to the lack of information about scabies disease in the west of Iran and the numerous reports of this disease, this study was to clarify the disease status. This investigation is a descriptive, cross sectional study. The disease was diagnosed based on clinical examination and microscopic observation of the parasite in the ulcer site from March 2006 to March 2011 referrals to health centers in Hamadan and Kermanshah provinces. The prevalence of scabies in Hamadan and Kermanshah provinces referred to health centers were determined approximately 0.0005. All cases of scabies in Hamadan and Kermanshah provinces is 177 peoples, that 170 (96%) of Kermanshah Province, and 7 patients (4%) is in Hamadan province. 73 (41.2%) of patients were illiterate and 104 (58.8%) had primary education or higher. 26 patients (14.7%) to 4 persons in households, and 151 (85.3%) in households of 5 persons or more are diagnosed with the disease. 104 (58.7%) of patients in the near or far from the place of residence had domestic animals, and 73 (41.3%) had no livestock. The housing situation of people with gall disease in 100 patients (56.5%) had inadequate housing, 75 (42.4%) with proper brick houses and 2 patients (1.1%) had a shed. Health education (personal hygiene and daily cleaning) and identifies those infected (quarantine and treatment) to reduce contamination to be removed from this disease is an important step.

Keywords:*Scabies, Sarcoptes scabiei, pruritus, Epidemiology*

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Abundance and dominance of sand fly species (Diptera: Psychodidae: Phlebotominae) in Zahedan County, Sistan - Baluchistan Province, Southeastern Iran

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Phlebotomine sand flies are the vectors of various types of leishmaniasis in Iran . In order to understand the faunal composition of sand flies , this survey was done in Zahedan county . A total of 13 cities an villages were screened for sand flies . Collections were made in 1997 ? 1998 . The specimens were collected by sticky paper traps coated with castor oil . The specimens collected were preserved in alcohol and slides were prepared in Puri's medium . In this study 6323 specimens were collected . We found nine species of Phlebotomus and thirteen species of Sergentomyia . P . papatasi (23.5%) , S . tiberiadis (17.7%) , S . clydei (14.2%) , S . sintoni (12.9%) , P . sergenti (9.1%) , P . alexandri (7.9%) and P . kazeruni (6.7%) were the predominant Phlebotomine species in our investigation . During the collection of sand flies , the species of , P . bergeroti , P . halepensis , P . keshishiani , P . major , S . africana , S . baghdadis , S . clydei , S . dentata , S . dreyfussi , S . grekovi , S . hodgsoni , S . iranica and S . sumbarica were collected for the first time from this county. In this study P . papatasi had highest density in Zahedan county . High density of P . papatasi and P . sergenti in this district could indicate risk of spread cutaneous Leishmaniasis.

Keywords:*Leishmaniasis, Sand fly, Fauna, Abundance, Zahedan County, Iran.*

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Medical Entomology

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Cockroaches [*Periplaneta americana* (L.), Dictyoptera; Blattidae] as carriers of bacterial pathogens, Khorramshahr County, Southwestern Iran

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American cockroaches are found in association with human dwellings and hospitals. They have a worldwide distribution. These domestic pests affect human health in several ways. Their habits make them ideal mechanical carriers of different pathogenic microorganisms. Numerous bacteria of medical importance have been isolated from cockroaches. The objective of this research was to determine the role of American cockroaches as carriers of pathogenic bacteria. This was accomplished through the isolation and identification of these microorganisms from the external surfaces of cockroaches captured in Health and Medical Services Centers and their surroundings. Seventy-three cockroaches were caught in Health and Medical Services Centers in Khorramshahr County, southwestern Iran, in 2006. The fluid used to wash the external surfaces of cockroaches was cultured to isolate and identify bacterial pathogens. Pathogenic bacteria were isolated from the external surfaces of 100% of the American cockroaches examined. The following bacterial pathogens were recovered from their body surface:Klebsiella (47.9%), Pseudomonas (37%), Escherichia coli (30.1%), Staphylococcus (24.6%), Enterobacter (19.2%), Streptococcus (15.1%), Serratia (8.2%), Bacillus (4.1%), and Proteus (2.7%). The bacterial pathogenic flora isolated from this cockroach species indicate that domestic pests could pose a health problem to humans. Thus, we must control cockroaches, particularly in indoors, sewage and solid wastes .

Keywords:*Bacterial Pathogens, Cockroach, Khorramshahr County, Iran*

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Data on sand fly fauna (Diptera, Psychodidae, Phlebotominae) in Khash County, Sistan - Baluchistan Province, Southeastern Iran

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Sand flies are the biological vectors of leishmaniasis all around the word. In 1997, sand flies were collected in 14 cities and villages of Khash County, using 848 sticky traps. A total of 4,673 sand flies were collected mainly from mountainous areas.The following 21 species were found : Sergentomyia tiberiadis (23.6%) , Phlebotomus kazeruni (24.4%) , P . sergenti (18.1%) , P . alexandri (12.9%) , P . papatasi (6.1%) , S .sintoni (4.9%), S . hodgsoni (3.9%) , S . baghdadis (1.7%) , S . dentate (1.2%) , S . mervynae (1.1%) , S . clydei (1%) , S . christophersi (0.3%) , P . eleanorae (0.2%) , S . dreyfussi (0.1%) , P . bergeroti (0.1%) , P . halepensis (0.08%) , S . iranica (0.06%) , S.theodori (0.06) , S . africana (0.04%) , P . major (0.04%) and P . mesghali (0.02%) . The following 14 species were reported for the first time from Khash County: P. papatasi, P .bergeroti, P. eleanorae, P. halepensis, P. major, P. mesghali, S. hodgsoni, S. mervynae, S. dreyfussi, S. iranica, S. theodori, S. africana, S. clydei and S. christophersi. The composition of species in Khash county is almost similar to the other parts of Iran. However, the dominance of P. kazeruni in Khash County may suggest that this species should be considered as a potential vector in the region of Khash.

Keywords:*Fauna, Sand fly, Khash County, Iran*

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American cockroaches [*Periplaneta americana* (L.), Dictyoptera, Blattidae) as potential vectors of nosocomial infections, Ahwaz City, Khuzistan Province, Southwestern Iran

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American cockroaches act as a main mechanical vector for many pathogenic bacteria . These agents can stay alive for a few days on external parts of cockroach's body . The aim of this research was to confirm that P . americana carry pathogenic bacteria in some hospitals. Fifteen cockroaches were catched from different wards of three hospitals (namely Arya , Sina and Amir ? Almohmenin) of Ahwaz city using direct collection , during 2006 . Collected cockroaches were identified according to systematic keys . The washing fluid from external surface of cockroaches were cultured for identify bacteria according to standard microbiological procedures . Results of culture media showed that 100% of cockroaches were positive to the bacteria . Among three hospitals it is found that Sina hospital was more infected . All the American cockroaches were infected to at least one bacterium . The main common bacteria was found Escherichia coli at which 86.7% of cockroaches were infected . The second common diagnosed bacterium was found to be Proteus vulgaris at which 73.3% external infection by this bacterium was reported . The least contamination was approved to Staphylococcus vulgaris . Only 6.7% of cockroaches were externally infected . In addition to these bacteria , Bacillus cereus (66.7%) , Streptococcus faecalis (60%) , Staphylococcus aureus (60%) , Enterobacter cloacae (53.3%) , Shingella (33.3%) and Serratia (13.3%) were isolated on cockroaches . Results exhibited that the presence of American cockroaches in the hospitals can threaten the health of hospitalized patients.

Keywords:*American Cockroach, Nosocomial Infection, Bacteria, Ahwaz City, Iran*

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A survey on species of isolated bacterial agents from american cockroaches (*Periplaneta americana*) in residential areas of Ahwaz City, Khuzistan Province, Southwestern Iran

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American cockroaches are often found in close association with humans, living in human places . Because of their association with human dewellings, cockroaches may incidentally contact and acquire human pathogens. The aim of this descriptive laboratory study was to assess the role of American cockroaches as potential carriers of pathogenic bacteria. This study was conducted in 2008 . A total of 25 American cockroaches were collected using hand catch method from houses. The cockroaches were transferred to clean tubes. Samples were transported to the laboratory. Then the cockroaches killed and identified. Bacteria on the body of cockroaches were isolated and identified with using the standard isolation techniques. Different species of bacteria were associated with cockroach. The bacteria showed various cultural, morphological and biochemical features. Medically important bacteria were isolated from external surface of 100% of cockroaches . Eleven bacterial species were isolated from cockroaches. The most common detected bacteria were found to be Escherichia, Staphylococcus aureus, Proteus, Klebsiella, Citrobacter and Enterobacter at which 100%, 72%, 60%, 60%, 56% and 52% of cockroaches were infected, respectively. The finding of E.coli among cockroaches means that these insects have been in contact with human faeces or faeces contaminated tools. The minimum contamination was approved to Serratia (20%), Micrococcus (32%) and Enterococcus (40%). The other extracted bacteria in this research were Psudomonas (44%) and Sterptococcus (48%) It is necessary to control these insects for providing a clean and healthy place. Application of low risk pesticide will reduce the harmful health effect of cockroaches .

Keywords:*American Cockroaches, Bacteria, Residential Environments, Ahvaz City, Iran.*

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An epidemiological study of *Pediculus capitis* infestation in the Cases of Attending to the Azna Health Centers, Lorestan Province,Western Iran

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Head louse, Pediculus capitis, is an obligatory ectoparasite with a worldwide distribution. This descriptive study was conducted on the cases with head lice infestations that attended to the Azna Health Centers and were examined by the trained health staff, during 2007. Suspected hairs of head were examined for nits and lice. Epidemiologic information of patients were recorded and collected in the questionnaires. Recorded data in the questionnaires were analyzed by SPSS software. The infestation of head lice was 95 cases . All infested cases were found among the females. The incidence of pediculosis was greater in the villages (70.5%) than in the urban (29.5%) areas. The prevalence rate of pediculosis among the age groups of <6, 6-10, 11-17 and >17 years old were 10.5% , 62.1% , 24.2% and 3.2%, respectively. Eighteen cases (18.9%) of patients had infestation background to pediculosis and 77 cases (81.1%) were without infestation background. The frequency of nit infestation was 90 cases and with adult and nymph were five cases. Remarkable percentage of patients (78%) complained from head itching. Seventy cases of patients were observed among the people who have used of common tools such as combs and towels. Most of the patients (79%) were without healthy bathroom at home. About 75.8% of patients had elementary knowledge. As the data of this study showed, head louse is an important health problem in Azna county, especially among the people with low education, who live in the area with poverty, low health and overcrowded conditions.

Keywords:*Epidemiology, Pediculus capitis, Health Centers, Azna County Iran*

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Epidemiological and clinical characteristics of scorpion sting in Bagh-e-Malek County, Khuzistan province, Southwestern Iran

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Forty to fifty thousands of scorpion sting cases and at least 7-60 death cases are reported annually from Iran. Around 25000 sting cases and several death cases are reported annually from Khuzistan province, where Hemiscorpius lepturus sting is responsible for 95% of stings. According to scorpionism importance in Bagh-e-Malek County, this cross sectional - descriptive study was conducted in 2007-2008 . Required informations were drawn from patient's files and reported in a questionnaire that was prepared for this purpose. After coding, the data were analyzed by spss software. This study was conducted on 524 victims . Totally, 363 (69.3%) patients were living in rural areas. 302 (57.6%) of sting cases were among women The most cases were belonging to age groups of 15-24 and 25-34 years old with 28.4% and 23.8% abundance, respectively.The most sting cases have occured in summer (46.8%) . Nearly, 214 patients (40.8%) were stung on the hand and 210 (40.1%) on the foot. The highest (77.2%) stings have occured at night . 45.4% of studied cases were stung at 0-6 . About 83% of patients were stung in indoors. The most prevalent symptoms of scorpionism were: pain of sting place (72.7%), irritation and wound (15.6%), severe muscle pain (7.8%) and numbness of sting place (3.9%). Patient's care, faster use of antivenum in emergency cases and recommended to be relax can be very effective in saving lives. Provide the necessary training for the families about safety principals observance is very useful to decrease the scorpion sting.

Keywords:*Epidemiology, Scorpion Sting, Bagh-e-Malek County, Iran*

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Insecticidal activity of *Moringa oleifera* seed extract against a dengue vector, *Aedes aegypti* L. (Diptera: Culicidae) in Faisalabad, Pakistan

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The larvicidal, pupicidal and repellent effect of the water extract of Moringa oleifera seeds was assessed against the dengue vector Aedes aegypti L. (Diptera: Culicidae). The bioassays were conducted under laboratory and in field conditions. Larvicidal effect of the treatments was assessed at different concentrations (0, 25, 50, 75 and 100 mg/L) after 12, 24, 36 and 48 h of the exposure against 2nd and 4th instar larvae, following the procedure of WHO with some modifications. The pupicidal activity was checked at same dose rates after 48 h of the treatments. The repellent activity of the extract was performed with human volunteers and dose-response method was used to estimate the percentage protection. The potential breeding habitats of the endemic areas of the district were sprayed and effect was recorded as percentage reduction in larval density. The results indicated significant effect of the treatments and the effectiveness increased with extended exposure interval with enhanced dose rates. The findings of the present study clearly revealed that water extract of M. oleifera seeds is an effective agent for the control of Ae. aegypti at its larval and pupal stage and exhibits good repellent action against adults, however, the efficacy depends upon dose rates and the exposure interval.

Keywords:*Dengue, mosquito, M. oleifera, seed extract, mortality*

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Contact and fumigant toxicity of *Pinus densiflora* needle hydrodistillate constituents and related compounds and efficacy of spray formulations containing the oil to *Dermatophagoides farinae*

Ju-Hee Lee[1], Jun-Ran Kim[2], Young Yull Koh[3], Young-Joon Ahn[4]

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The toxicity of red pine needle hydrodistillate (RPN-HD), 19 RPN-HD constituents and 12 structurally related compounds and control efficacy of four experimental spray formulations containing RPN-HD (0.5, 1, 2 and 3% sprays) to adult Dermatophagoidesfarinaewas evaluated. RPN-HD (24 h LC50,68.33 µg cm^2) was toxic. Menthol was the most toxic compound (12.69 µg cm^2) and the toxicity of this compound and benzyl benzoate did not differ significantly from each other. High toxicity was also produced by α-terpineol, bornyl acetate, geranyl acetate, thymol, linalyl acetate, terpinyl acetate, citral, linalool and camphor (18.79?36.51 µg cm^2). These compounds were more toxic than either deet or dibutyl phthalate. In vapour-phase mortality tests, these compounds were consistently more toxic in closed versus open containers, indicating that their mode of delivery was largely a result of vapour action. RPN-HD 3% experimental spray provided 95% mortality against adult D. farinae, whereas permethrin (cis:trans,25:75)2.5gL^-1 spray treatment resulted in 0% mortality. In the light of global efforts to reduce the level of highly toxic synthetic acaricides in indoor environments, RPN-HD and the compounds described merit further study as potential biocides for the control of Dermatophagoides populations as fumigants with contact action.

Keywords:*Dermatophagoides farinae, botanical acaricide, natural fumigant, Pinus densiflora, red pine needle hydrodistillate*

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Fumigant toxicity of plant essential oils against camptomyia corticalis (Diptera: Cecidomyiidae)

Jun-Ran Kim[1], Perumalsamy Haribalan[2], Bong-Ki Son[3], Young-Joon Ahn[4]

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The toxicity of 102 plant essential oils against third instars of cecidomyiid gall midge, Camptomyia corticalis, was examined using a vapor-phase mortality bioassay. Results were compared with that of a conventional insecticide dichlorvos. Based on 24 h LC50 values, all essential oils were less toxic than dichlorvos (LC50, 0.027 mg/cm^3). The LC50 of caraway seed, armoise, sage (Clary), oreganum, lemongrass, niaouli, spearmint, cassia special, sage (Dalmatian), thyme red, bay, garlic, and pennyroyal essential oil is between 0.55-0.60 mg/cm^3. The LC50 of cassia pure, thyme white, cassia redistilled, star anise, peppermint, wintergreen, cinnamon bark, majoram, chamomile romon, eucalyptus, rosemary, cedar wood, pimento berry, savory (summer), lavender, and coriander oil is between 0.61-0.99 mg/cm^3. All other essential oils tested exhibited low toxicity to the cecidomyiid larvae (LC50,>0.99 mg/cm^3). The active principles of active essential oils were identified by GC-MS analysis. The major active principles are such as pulegone, thujone, camphor, 1,8-cineole, a-pinene, thymol, menthol. At a rate of 5 mg/cm^3, pulegone, and thujone, exhibited 100% morality whereas camphor, 1,8-cineole, α-pinene, thymol and menthol showed 90% mortality at 15 mg/cm^3. Global efforts to reduce the level of highly toxic synthetic insecticides in the agricultural environment justify further studies on the essential oils and their constituents described as potential insecticides for the control of the mushroom fly as fumigants with contact action.

Keywords:*Cecidomyiid gall midge, Camptomyia corticalis, Essential oils, GC-MS, Cotanical insecticide, Natural fumigant*

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Transovarial transmission of *Orientia tsutsugamushi* in *Leptotrombidium palpale* (Acari: Trombiculidae)

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Tsutsugamuhi disease is transmitted through biting by unfed larval trombiculid mites infected with Orentia tsutsugamushi (Hayashi). It is a notorious vector borne disease in Korea. There were more than 5,000 cases annually since 2004. Leptotrombidium palpale was one of the vector mites of Tsutsugamushi disease in Korea. Transovarial transmission of O. tsutsugamushi was studied in two generations (P, F1) of naturally-infected L. palpale. When compared with uninfected mites, the naturally-infected L. palaple showed no significant difference in the oviposition rate but higher hatching rate. The uninfected female produced only uninfected progenies in both cases of mating with either an infected or uninfected male. However, the infected female vertically transmitted O. tsutsugamushi to its progenies in both case. The transovarial infection rates of parental and F1 generation was 100%. The overall filial infection rate in F1 generation was 100%, but this rate was declined to 95.3% in F2. This high rate of transmvarial transmission suggests that L. palpale might be one of major vector of tsutsugamushi disease in Korea.

Keywords:*trombiculid mite, transovarial transmission, scrub typhus*

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Seasonal distribution of chigger mites surveillance using new chigger mite collecting traps in Korea (2010~2011)

Won Il Park[1], Jong Yul Roh[2], Eun Hee Shin[3], Bong Gu Song[4], Chan Park[5], Mi-Yeoun Park[6], Jin Suk Han[7], Hyeon Joo Lee[8], E-hyun Shin[9]

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A surveillance of chigger mites was conducted to clarify the seasonal distribution of scrub typhus vectors by new chigger mite collecting traps from each 4 collection points of 2 regions from 2010 to 2011 in Korea. During the surveillance period, the population density of chigger mites had two peaks, one big peak in October-November and another small peak in March. In Hwaseong-city, a total of 185 mites representing 3 genera and 5 species were collected and the predominant species were Leptotrombidium pallidum (46.5%) and L. palpale (36.2%). In Goryeong-gun, a total of 1,397 mites representing 3 genera and 8 species were collected and the predominant species were L. scutellare (54.8%), L. pallidum (20.0%) and L. palpale (18.6%). The high collecting rates were recorded at field (92.8%) and at rice field (43.2%) in Hwaseong-city and Goryeong-gun, respectively. This result show that the surveillance of scrub typhus vectors by new chigger mite collecting trap is useful as an alternative for a conventional method by trapping wild rodents. but further research on increasing collection rate will be needed.

Keywords:*scrub typhus, seasonal distribution, chigger mite collecting trap, Leptotrombidium pallidum*

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Insecticide resistance monitoring in the principal Japanese encephalitis vector, *Culex tritaeniorhynchus* using bioassay and biochemical assays

Hyun Kyung Kim[1], Dae Hyun Yoo[2], Seong Yoon Kim[3], E Hyun Shin[4], Mi Yeoun Park[5], Kyu Sik Chang[6], Young Joon Ahn[7], Dong-Kyu Lee[8]

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Culex tritaeniorhynchus is the main Japanese encephalitis (JE) vector in the Republic of Korea (ROK). However, 26 of JE cases were reported in 2010 and vector control of JE should be need. Insecticide resistance of the JE vector mosquito has been one of major obstacles for effective control. In the ROK, few resistances of Cx. tritaeniorhynchus have been reported for the recent five years and accurate resistance status of the vector mosquito should be monitored for effective control. The insecticide resistances of field collected Cx. tritaeniorhynchus from Busan-si (designated BRCT population) and Jeonju-si (JRCT population) to 10 different insecticides were evaluated with an emulsion bioassay and an insecticide resistance related enzyme assay. Enzyme levels of acetylcholinesterase (AChE), non-specific esterases (NSE), glutathione S-transferases (GST) and mixed function oxidases (MFO) were evaluated with micro plate reader. A half of resistance ratio (RR50) values of BRCT population to all tested insecticides were 1.1-2.3 fold higher than those of JRCT population, except chlorpyrifos (RR=0.38). An enzyme activity between BRCT and JRCT populations in level diagram was significantly different and enzyme activities of BRCT population also were higher than the JRCT population, except β-NSE. These results allowed us to simply and effectively monitor the levels of resistance in filed collected mosquito populations with a smaller sample. Further studies on enzyme assay to evaluate resistant level of mosquitoes depending on a kind of insecticide should be need for effective insecticide resistance monitoring.

Keywords:*Culex tritaeniorhynchus, enzyme microplate assay, insecticide resistance, detoxifying enzyme*

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Population density and *Plasmodium vivax* infection rates of *Anopheles* mosquitoes in the malaria epidemic areas, Incheon-si, Gyeonggi-do and Gangwon-do, in the Republic of Korea

Tae Joon Lee[1], Sun Jae Bang[2], Myung-Deok Kim[3], E Hyun Shin[4], Kyu Sik Chang[5]

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The vector mosquito is one of important factors in transmission of vivax malaria. To effectively management vivax malaria, ecological monitoring of vector mosquitoes, such as distribution and vivax infection rates, must be need. Population density and vivax infection rates of vector mosquitoes were monitored in Incheonsi, Gyeonggi-do and Gangwon-do near Demilitarized zone (DMZ) in Northern part of the ROK with black light traps from April to October for 10 years, 2001 to 2011. The highest malaria cases were reported in 2006 and 2007 and a mean population density of vector mosquitoes during mosquito season in the years was higher than that of other years since 2001. However, in 2004 and 2011 malaria cases and density of vector mosquitoes were lower than those in other years since 2001. Population density of vector mosquito at monitoring areas in 2011 decreased over 80% than those for recent three years. The decrease of vector mosquitoes seem to be resulted from heavy rain and decrease of blood source by foot-and-mouth disease virus, Aphtae epizooticae, when considering data of Korea meteorological administration and Statistics Korea. High precipitation (mm) over 50% and increased rainy days during mosquito season may disturb blood feeding action of vector mosquitoes and expansion of population density. Plasmodium vivax infection rates of vector mosquitoes in Gangwon-do and Gyeonggi-do in 2011 more decreased as 0.12% and 0.36%, respectively, than those in the recent three years. Further studies on relationships among climate factors, vector density and patients should be need.

Keywords:*Malaria, vector mosquitoes, Plasmodium vivax, climate factors, the ROK*

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Tissue-specific promoter for the development of transgenic silkworm, *Bombyx mori*

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We have characterized that a sw17255 gene was expressed in hemocyte-specific tissues of the silkworm, Bombyx mori L. (Lepidoptera: Bombycidae). To determined core promoter region, 10 different partial mutant clones were tested by luciferase assay in the Sf9 cells. About 1.5 kb promoter shows higher luciferase activity then constitutive promoter (BmA3). To verify tissue-specific expression, we analyzed real-time quantitative RT-PCR using the highly expressed endogenous Actin RNA as an intrinsic reference. Here to determine the core promoter region, 4 different partial mutant clones were tested using the luciferase assay in the silkworm by direct injection of target plasmid DNAs. It was verified that the sw17255 core promoter region contains elements that regulate the expression of this gene in hemocyte tissue; the selected promoter region spans nucleotides -1 to -2,112 upstream of the start codon. The promoter candidate [E] (-579/-1) showed more than 1.67 fold transcriptional activity compared to control promoter activity. Development of this promoter directly or indirectly specifically inducible in the tissue is very useful to make recombinant proteins in the transgenic silkworm.

Keywords:*Tissue-specific, Promoter, Transgenic silkworm, Biomaterials*

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Functional genomics of yellow-g and yellow-g2 of the Asian tiger mosquito, *Aedes albopictus*

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Pigmentation or melanization is an important physiological event in insects and is involved in cuticle tanning, wound healing and encapsulation as a defensive response. Dopachrome-conversion enzyme (DCE, yellow) significantly accelerates the pigmentation/melanization reaction. A similar pigmentation/melanization process is used in the tanning (hardening and darkening) of the egg chorion in some mosquitoes. The goal of this study was to determine whether ovary-specific yellow genes (AalY-g and AalY-g2) are required for rigidity of the egg of the asian tiger mosquito, Aedes albopictus. Full-length of cDNAs for AalY-g and AalY-g2 were cloned and sequenced. Developmental- and tissue-specific expression patterns of these two genes were analyzed by RT-PCR and real-time PCR. AalY-g and AalY-g2 encode a protein of 379 and 373 amino acids containing a signal peptide. The transcripts of both genes were detected in the ovary of mature adult females only after blood feeding (48 to 72 h post-blood meal). When dsRNA for AalY-g or AalY-g2 was injected into blood fed-adult females, the treated females did oviposit. However, the outermost colorless exochorion of the eggs was fragile and partially peeled off. Some of the eggs (~5%) were not melanized and most of them delayed initial melanization (~3 h post-oviposition) of their endochorion. When the melanized-eggs (72 h post-oviposition) were placed in dry condition, approximately 50% eggs were collapsed. These results support the hypothesis that Yellow-g and Yellow-g2 are critical in chorion tanning, vitelline membrane hardening and desiccation resistance of mosquito eggs.

Keywords:*Aedes albopictus, yellow, pigmentation, melanization, chorion, vitelline membrane*

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Predation and control efficacies of *Misgurnus mizolepis*(Cypriniformes: Cobitidae) toward *Culex pipiens molestus* (Diptera: Culicidae) and fish toxicity of temphos in laboratory and septic tank conditions

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An assessment was made of the biological control potential of mud loaches, Misgurnus mizolepis Gunther (Cypriniformes: Cobitidae), toward Cx. pipiens molestus larvae, in laboratory and septic tanks. Results were compared with those of temephos 20% emulsifiable concentrate (EC). In the lab tests, all mud loaches survived on sedimentation chamber and effluent chamber water of aerobic septic tanks (ASTs), whereas all mud loaches died within 3?12 h after introduction into sedimentation chamber and effluent chamber water of anaerobic septic tanks (AnASTs). Gill hyperplasia and hemorrhages at the bases of pectoral fins were detected in all dead mud loaches. These appear to be caused by bacterial disease because the physical and chemical parameter values of the AST and AnAST water were highly variable. A mud loach consumed an average of 951?1,087 larvae of Cx. p. molestus in the AST water at 24 h. The temephos formulation did not cause fish mortality at a company recommendation rate (10 ml/ton) in the AST water. In the AST experiment, predation of mosquito larvae by mud loaches at a release rate of 900 mosquito larvae/fish resulted in complete mosquito control from the 3rd or 7th d after treatment (DAT) through the end of the survey period for 18 wk, compared with two temephos 20% EC-treated AST water (reduction rate, 41 and 33% 28 DAT). Mud loaches merit further study as a potential biological control agent for the control of mosquito populations in septic tanks.

Keywords:*Misgurnus mizolepis, Culex pipiens molestus, biological control, mosquito control, predation, septic tank*

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Molecular detection of tick-borne pathogens in ixodid ticks from Pakistan

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Ticks are an example of efficient ecto-parasites that blood-feed on variety of hosts including humans, domestic and wild animals. Ticks transmit a variety of bacterial, protozoan, and viral disease agents during blood feeding on the host. The aim of this study was to evaluate tick-borne infection in ticks from different ecological regions of Pakistan infesting ruminants. Ticks (?574) were collected from ruminants and identified to species level. The ticks were identified as Hyalomma anatolicum anatolicum, Hyalomma dromedarrii, Rhipicephalus microplus, Rhipicephalus sanguineus, and Argus persicus. The prevalence of Spotted Fever Group Rickettsia (SFGR), Babesia, and Theileria wwere examined using Polymerase Chain Reaction (PCR). The nucleotide sequence similarity of the PCR products revealed infection of Theileria, Babesia, and SFGR species. Additionally, the results of pathogen-load and the microbial community residing in single H. anatolicum anatolicum and R. microplus by using 454-based pyrosequencing approach will be discussed.

Keywords:*Hyalomma anatoliculum, Rhipicephalus microplus, Babesiosis, Spotted Fever Group Rickesttsiosis*

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Evaluation of two storage mites life tables on dried oyster mushroom and their harmful effects at different constant temperatures

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The current study aimed to estimate the influence of three constant temperature (20 ºC, 25 ºC and 30 ºC) on the population growth, damage effects and life table of two stored product mites (Tyrophagus putrescentiae and Aleuroglyphus ovatus) reared on oyster mushroom at 80 % relative humidity (R.H.) and a photoperiod of 14 light: 10 dark (L: D). The rate of the increase (r) was (0.161 and 0.111), (0.175 and 0.122) and (0.154 and 0.094) at 20, 25 and 30 °C due to the effect of T. putrescentiae and A. ovatus, respectively. The highest decreases in the weight of oyster mushroom were 18.23 % and 12.50 % at 25 ºC in the presence of 320 individuals of T. putrescentiae or A. ovatus, respectively. The female longevity of T. putrescentiae and A. ovatus varied from 34.74 and 45.37days at 20 ºC to 23.17 and 31.16 days at 25 ºC and to 17.55 and 24.44 days at 30 ºC, respectively. The results also showed that net reproductive rate (R0), population doubling time (PDT) and Mean generation time (T) for T. putrescentiae and A. ovatus affected negatively as temperatures increased. In contrast, both intrinsic rate of increase (rm) and Finite rate of increase (λ) increased as results of temperature decreased. The highest rm of T. putrescentiae (0.265) and A. ovatus (0.140) were noticed at 25 ºC. Conversely, the lowest rm for T. putrescentiae (0.171) and A. ovatus (0.09) were recorded at 20 ºC.

Keywords:*life tables, storage mites, harmful effects, oyster mushroom*

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Predictors of dengue fever and dengue haemorrhagic fever in Punjab, Pakistan

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Purpose of study: This study was conducted to assess the future threat of Dengue Fever and Dengue Haemorrhagic Fever in Punjab, Pakistan Type of Study: Prospective descriptive study. Place and duration of Study: Primary data of laboratory confirmed cases was collected from all levels of health facilities of public and private sector. Disease vectors were collected from Lahore. Data of rainfall and temperature were obtained from website of department of meteorology Pakistan to determine the potential values. Methodology: Vector-borne diseases are the serious health problems worldwide, including Pakistan. Coverage of control interventions and physio-environmental factors were also analyzed. Results: In 2008 there was a largest epidemic of dengue fever in Punjab and 1382 cases were reported in 16 districts out of 36, which increased in number of cases up to 5724 and district up to 34 in 2010. Our results suggest that rainfall and temperature were predictive of the abundance of Ae.aegypti and Ae.albopictus and in time interventions have positive significant impact on disease transmission. Conclusion: This paper offered useful information that the climatic and physio-environmental factors like built-up, agriculture, water bodies and forest areas have different influence on the dengue fever incidences. This will help in focusing the preventive measures being applied on priority in very high and high-risk zones and save time and money. No special funds were used for this study.

Keywords:*Ae.aegypti, Ae.albopictus, precipitation, temperature, prediction, physio-environment.*

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Daily survival, anthropophily and the role of major *Anopheline* vectors in malaria transmission in South west Nigeria

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To evaluate the vector efficiency of Anopheles gambiae s.s, An.arabiensis and An.funestus, An.moucheti and An.nili in transmitting falciparum malaria in three villages in South west Nigeria, daily survival and feeding host preferences were studied between January 2000-December 2003, and July?December 2004. Mosquitoes were collected by human?landing catches (HLC) and pyrethrum spray catches. Morphological and molecular characterisation, host blood meal preferences, parity and daily survival, sporozoite and entomological inoculation rates assessment of the anophelines were performed. A total of 20393 adult female anophelines belonging to twelve different species were caught. Out of these, the major vectors; Anopheles gambiae s.s, An.funestus, An. arabiaensis, An.nili and An.moucheti constituted 77.83%, while the secondary vectors comprised only 22.17%. There was a substantial indoors to outdoor variation and seasonality in the distribution of the Anopheles population. All the five species showed similar nocturnal biting patterns and were sympatrically involved in malaria transmission simultaneously or replacing each other seasonally. The mean anthropophagic tendency of the major malaria vectors was high with the mean HBI >75%. The annual mean parous rate of 59.15% was recorded among the major vector species with the highest rate of 76.96% in An.funestus population and the least rate of 43.07% in An.nili. Mean probability of daily survival of malaria vectors was 0.76, with annual mean life expectancy of 13.85days and the expectation of infective life was short with a mean of 4.79 days. The average infection and Entomological inoculation rates, among the major anophelines were 6.52% and113 bites/person/year, respectively.

Keywords:*Malaria vectors, Daily survival, entomological inoculation rates, Parous rates, life exp*

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The effect of oxidative stress on the DDT insecticide resistance phenotype and life history of the major Malaria vector *Anopheles arabiensis*

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The upregulation of oxidative stress related transcripts has been described in wild, bloodfed Anopheles arabiensis, but no further studies have been reported on the relationship between oxidative stress and insecticide resistance. This study examines the effect of oxidative stress on the expression of DDT resistance and life history of laboratory-reared An. arabiensis. The effect of multiple bloodmeals on DDT resistance was evaluated by WHO bioassay. The effect of protein supplementation was examined by allowing DDT susceptible and resistant adult An. arabiensis continuous access to 10% sucrose supplemented with 3.4x 10-4 µM recombinant human insulin and measuring their longevity. To examine the effect of the insulin on DDT resistance, adults were bioassayed after three days access to 5.9x 10-4 µM recombinant human insulin. The difference in the capacity of susceptible and resistant strains to cope with insecticide and superoxide generated oxidative stress was determined by quantifying oxidative stress markers and the activity of oxidative stress relieving enzymes. Protein supplementation in the form of insulin significantly decreases the lifespan of DDT susceptible, but not resistant, females. It also results in a small but significant decrease in DDT resistance despite the presence of a knockdown resistance (kdr) genotype. Multiple bloodmeals cause a significant increase in DDT resistance after 15 days, suggesting that females that have taken multiple bloodmeals are more likely to survive DDT intoxication at the critical age for Plasmodium transmission. This is one of the first studies to report the biochemical effects of oxidative stress on insecticide resistance.

Keywords:*insecticide resistance, oxidative stress*

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Effect of Mohlolo bait on age- related *Blatella germanica* population levels

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The control strategies of cockroach populations have shifted from predominant use of insecticide sprays to the inclusion of baits in management programmes. The effect of Mohlolo bait on German cockroach populations was investigated in SOuth Africa, where the pest has attained uncontrollable status in homes.The study was conducted at Limpopo Agro-Food Technology Station (LATS) laboratory, University of Limpopo. The laboratory was maintained at 24-28d egree Celsius, ambient humidity, and a photoperiod of 10: 14 (L: D).A complete randomized design was used, with age of cockroaches as treatments. The experiment comprised of six treatments which were 1-d old, 2-d old, 3-d old, 4-d old, and 5-d old German cockroach nymphs. Mortality was recorded daily (until all the nymphs were dead. The data was subjected to the analyses of variance by using Statistics 8.1 software. Mean separation for significant treatment was achieved using the Tukey's multiple range tests at 5 % level of significance. The period of exposure to Mohlolo bait attributed 97%, 99%, 100%, 100% and 100% of total variation in 1-d, 2-d, 3-d, 4-d and 5-d old nymphs respectively, The study showed that 1-d old B. germanica nymphs are more resistance to Mohlolo bait compared to 2-d, 3-d, 4-d and 5-d days old B.germanica nymphs. According to the data, 100% mortality of 1-d old nymphs was obtained after seven days exposure to Mohlolo bait, whereas the subsequent ages could not live beyond 4 days.

Keywords:*Blatella germanica, mohlolo bait*

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Field evaluation of BG-Sentinel and BG-Mosquitito traps for filarial vector *Aedes polynesiensis* in French Polynesia

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The efficacy of the BG-SentinelTM and the BG-MosquititoTM traps was evaluated in French Polynesia against human bait catch using the CDC Backpack aspirator. Efficiency was measured by comparing collection methods in a randomized Latin-square design primarily for collection of host-seeking females of the filarial vector Ae. polynesiensis. Production of CO2 from yeast-sugar fermentation was measured (average 105 ml/min) and proved to be a suitable alternative source of CO2 where either dry ice or gas cylinder was unavailable. The various collection apparatus were tested on two typical islands of French Polynesia: the high, volcanic island of Moorea, and the low, coral island (atoll) of Tetiaroa. On Moorea, efficiency of the BG-Sentinel traps baited with either BG-lure or CO2 plus octenol were significantly more efficient than the BG-Mosquitito with BG-lure. Catch rates of BG-Sentinel or BG-Mosquitito baited with either BG-lure or CO2 plus octenol did not differ significantly to the CDC Backpack aspirator. On Tetiaroa, the operation of BG-Mosquitito was greatly affected from interference caused by rodents and consequently these traps were removed from comparison. Catch rate of the BG-Sentinel with either BG-lure or CO2 plus octenol did not differ significantly to the CDC Backpack aspirator. Both BG-Sentinel and BG-Mosquitito trap proved to be suitable efficient and convenient alternatives to the landing catch collection method for sampling Ae. polynesiensis. However, the BG-Mosquitito's poor reliability in the field and the lower quality of mosquito specimens resulting from this trap demonstrate that the BG-Sentinel is a more adapted tool for Aedes mosquito field research.

Keywords:*BG-Sentinel, BG-Mosquitito, Aedes polynesiensis, French Polynesia*

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Entomological characteristics after five years of malaria control on Bioko Island, *Equatorial Guinea*

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The Bioko Island Malaria Control Project (BIMCP) in Equatorial Guinea started in 2004 with the goal to reduce malaria transmission and associated morbidity and mortality on Bioko Island. In 2009 the project was extended by a second 5-year term from 2009-2013. To achieve these goals a set of integrated interventions were implemented combining vector control, effective case management, improved management of malaria during pregnancy, public communication, monitoring and evaluation, and operational research. Vector control consists of indoor residual spraying (IRS) with bendiocarb and long-lasting insecticide treated bed-nets (LLIN). Here we report the results from the human landing collections of 2009 on vector density, biting times, species composition, sporozoite rates, and entomological inoculation rates (EIR). Regular adult overnight mosquito collections were carried out by human landing collections at three sites on the island. In the northwestern Punta Europa area high human biting rates of Anopheles mosquitoes and high sporozoite rates produced an EIR of 883, the highest on the island. In this area about 100% were Anopheles gambiae s.s. The EIR in southeastern Riaba was 249 (46% An. gambiae s.s. and 54% An. melas) and in southwestern Arena Blanca 145 (almost 100% An. melas). Biting was more prevalent outdoors. Large spatiotemporal variations in mosquito density, biting activity, species composition and sporozoite rates indicate that pockets of high malaria transmission remain despite a largely successful control program. These hotspots require additional specific targeted vector control interventions, e.g. larval control or environmental management.

Keywords:*malaria, vector control, Central Africa, entomological inoculation rate*

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Natural product of Yartsa Gumba an entomo-fungal complex of Indian Himalayas

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ABSTRACT Yartsa gumba or Yar-rtsa-dgun-bu is Tibetan name of an Entomo-Fungal combination between Hepialus sp. (Lepidoptera; Hepialidae) larvae and its parasitizing fungus (Cordyceps sinensis), which is traditionally used in Tibetan and Chinese System of Medicine (TCM). This medicinal entomo-fungal product is known in Chinese as Dong Chong Xia Cao (winter worm and summer plant or grass in summer and worm in winter) Yartsa Goenbub in Bhutan, Yarchagumba means herbs of life in Nepal and Tochukaso in Japan. It is also known as Caterpillar mushroom, Caterpillar fungus, vegetable wasp, plant worm, vegetable caterpillar (Cooke,1892) and in India it is commonly known as Keera Ghas. This entomo-fungal combination is known to be used for many centuries as tonic, medicine and aphrodisiac and in religious ceremonies in China, Indonesia and Upper Himalayas. The Importance of this combination is due to a fungus viz Cordyceps sinensis parasitizing the host caterpillars (Arif and Kumar 2003). In nature host (Hepialus) and parasitizing fungus (Cordycep)s both occur in difficult terrain of high altitude areas ranging from 10500-16000 feet altitude and are rare. It occurrence in high mountains at an altitude above 4000 m was noticed and reported by Garbyal 2000 & 2001. Exhaustive work has been done on high altitude insects and its biology by various workers (Mani 1995, Arif 1997, Arif & Kumar 1995, and Arif and Kumar 2002) Native occurrence of the fungus is confined to high Himalayan mountains in Tibet, Nepal, China, India, Bhutan and Sikkim at an altitude ranging from 3000-5000 m amsl.

Keywords:*YARTSA GAMBU,ENTOMOFUNGAL,MEDICINES*

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Field efficacy of peridomestic insecticide space spraying against two *Aedes* populations in Tahiti, French Polynesia

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In the absence of specific treatments or vaccines, vector surveillance and control are the only measures available to suppress vector borne disease transmission. In French Polynesia, current public health interventions are based mostly on the use of chemical insecticides. The effectiveness of vehicle-dispensed peridomestic space spraying of insecticides in reducing Aedes populations was investigated in the rural village of Toahotu, Tahiti. Mean 24 hr Aedes catches using BG-Sentinel™ traps were used to evaluate the outcome of permethrin space spraying. Outdoor entomological sampling was performed before and after a single insecticide ULV application at a control and treatment site. No significant differences were observed in Aedes trap catches between control and treatment during the 3 weeks preceding the insecticide application. Immediate reduction of Aedes polynesiensis females was observed after spraying with mean trap catches significantly different between treatment and control sites. However, this difference was only transient with adult female population returning to pre-spray level after 4 to 7 days post-treatment. For Aedes aegypti, no significant difference was observed between treatment and control after spraying. A significant increase in Aedes aegypti female trap catches was observed at the treatment site 4-7 days after ULV application. Altogether, our results indicate that the effectiveness of peridomestic space spraying is very limited in time and particularly unsuitable for the control of the (indoor) population of Ae aegypti.. Our results support the development and field evaluation of innovative, more efficient, and more sustainable control methods to reduce the risk of dengue and other arbovirus transmission.

Keywords:*vector-borne diseases, dengue, chikungunya, Aedes aegypti, Aedes polynesiensis, BG-Sentinel™, insect*

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Prey preference of aquatic insects: Implications in regulation of wetland mosquitoes

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Wetlands are potential sites for mosquito breeding and are thus important from public health viewpoint. Chemical and microbial controls are constrained in wetlands due to prospective impact on the diverse biota. Biological control using generalist aquatic insects can serve the purpose, provided a preference for mosquito larvae is exhibited. Mosquito prey preference of three water bugs and larvae of two odonate species was evaluated using chironomid larvae, fish fingerlings and tadpoles as alternative prey. Manly's selectivity (αi) value with 95% confidence interval (CI) was estimated to judge the prey preference pattern. MANOVA and standardized canonical coefficients were used to test the density effects on the prey selectivity. The αi values indicated significant preference ($P < 0.05$) for mosquito by all the insect predators against the alternative prey as a density dependent function. On a comparative scale chironomid larvae had highest impact as alternative prey. In multiple prey experiment, the predators showed a similar preference pattern of mosquito over the alternative prey reflecting a significant ($P<0.05$) niche overlap. The results suggest that these predators can effectively reduce mosquitoes in presence of multiple alternative prey. Their use in biocontrol of mosquitoes of wetland will enhance conservation and meet the requirements of public health.

Keywords:*mosquitoes, wetlands, vector control, alternate prey*

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Insecticide resistance in *An. Arabiensis* from Sudan: Temporal trends and underlying mechanisms.

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Insecticide resistance is a real threat to the sustainability of any malaria vector control program in Africa. Malaria vector control in Sudan relies mainly on indoor residual spraying (IRS) and the use of LLINs. Studies on the molecular mechanisms conferring insecticide resistance are important in both understanding the molecular basis of resistance and to minimising its impact on disease control. WHO susceptibility tests were carried out to detect resistance to DDT, fenitrothion, bendiocarb, permethrin and deltamethrin. Molecular assays were carried out to determine kdr allelic frequencies. The gene-expression profile of a highly permethrin-resistant population of An. arabiensis from central Sudan was characterized using a unique whole-genome microarray. Throughout the surveys An. gambiae s.l. populations from all sites were fully susceptible to bendiocarb and fenitrothion. However, high frequencies of resistance to DDT and pyrethroids were seen in three sites. This study reported seasonal variations of insecticide resistance from all sites. The 1014F kdr allele was present in all four populations tested and the presence of this allele was significantly correlated with resistance to pyrethroids and DDT ($P < 0.05$). The 1014S allele was not detected in any of the populations tested Two cytochrome P450s were significantly over expressed in the survivors, CYP6 Z3 (10.2 fold) and CYP6P3 (6.7 fold). This is the first study to demonstrate P450s in An. arabiensis from Sudan. The findings add to the understanding of the genetic basis of insecticide resistance in wild mosquito populations from Sudan.

Keywords:*An. arabiensis, insecticide resistance, central Sudan, 1014F kdr, cytochrome P450s*

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Contact toxicity of *Lavandula angustifolia* Mill and *Zataria multiflora* Boiss essential oils against *Callosobruchus maculatus* adults (Coleoptera: Bruchidae)

Gholamhossein Moravvej[1], Zahra Golestani Kalat[2], Arezoo Heydarzade[3]

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In last decades, many researches have been down on use of botanical compound especially essential oils as insecticides. In the present study, the contact toxicity of Lavandula angustifolia and Zataria multiflora essential oils were evaluated on both sexes of 1-2 day-old C. maculatus adults at 30±1ºC, 60±5% RH and dark condition. Essential oils were obtained by hydrodistilation. The toxicity of oils against males and females were positively and significantly associated with concentration. On the basis of LC50 ratios and their 95% confidence limits, there was no significant difference between sensitivity of males and females. The LC50 values were 719 and 598 µl.m-2 against males and 787 and 738 µl.m-2 against females for L. angustifolia and Z. multiflora, respectively. Both oils showed no toxicity on cowpea (Vigna unguiculata) seed germination. Based on the analysis by GC-MS, the main compounds of L. angustifolia oil were linalool (42.8%), 1,8-cineol (23.4%), rosefuran epoxide (14%), menthone (6.8%), isomenthol (5.2%) and dihydro carvone (trans) (4.3%) and those of Z. multiflora oil were thymol (55%), linalool (37.8%) and p-cymene (7.2%). The results suggested that L. angustifolia and Z. multiflora essential oils might be suitable alternatives to conventional pesticides for protecting pulses against C. maculatus adult.

Keywords:*Essential oils, Lavandula angustifolia, Zataria multiflora, Contact toxicity, seed germination*

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The first report of the aphid *Cinara pini* from Iran and study on its morphology and biology

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During a survey on pine Pinus mugo Turra pests in April 2010 in Mashhad, the aphid species of Cinara pini (Linnaeus, 1758) was observed in abundant populations particularly on the seedlings. The aphid belongs to the subfamily Cinarinae and family Lachnidae. The mean adult size was 2.5 to 2.9 mm. The base of final antennal segment has fewer than 4 hairs. The final rostrum segment is longer than the basal diameter of cornicles or equals to or slightly longer than the second segment of hind leg tarsus. The aphid had three nymphal instars lasting 10 days in greenhouse conditions (25±2 °C, 45±5 % r.h.). The period from adult emergence until first reproduction lasted 48 hours. The reproductive period lasted 13 days during which a mean of 36 nymphs were deposited. The total length of one generation was 25 days.

Keywords:*Cinara pini, Morphology, Phytophagous, Pinus mugo*

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Molecular mechanisms of insect diapause: Supplementing alanine enhances cold tolerance in non-diapause *Calliphora vicina* larvae

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Winter provides a period of extreme cold and limited resource availability. To survive winter many temperate insects enter diapause, a period of reduced metabolic activity. Diapause synchronises development with favourable conditions, and is associated with an enhanced ability to tolerate cold exposure. In the blowfly, Calliphora vicina, non-diapause larvae freeze (SCP) at -11.7+1.3°C and display 50 % mortality (LT50) after 5.1 days at -4 °C. Diapause larvae have an enhanced ability to tolerate the cold, with a mean SCP of -18.9+0.6°C and LT50 after an astonishing 42.1 days at -4°C. The physiological responses of diapause are well understood, but little is known about the underlying molecular mechanisms. A metabolomic analysis identified diapause and non-diapause larvae to follow distinct development pathways; with metabolites such as alanine and proline up-regulated during diapause. Alanine stabilises enzyme structure and function at low temperatures, and proline is known to increases cold tolerance in many species. Supplementing diets of non-diapause C. vicina with alanine and proline allowed us to identify what role these amino acids have in inferring cold tolerance. Non-diapause larvae supplemented with an alanine diet displayed a lowered SCP of -13.6 +1.0 °C and LT50 of 15 days at -4 °C, an increase in cold tolerance when compared to non-supplemented larvae (SCP of - 11.7+ 1.3 °C and an LT50 of 5.1 days). However, supplementing with proline failed to enhance cold tolerance. In conclusion, diapause represents a distinct development pathway, in which alanine has an important role in ensuring survival during the winter period.

Keywords:*diapause, metabolites, cold tolerance, alanine*

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Effect of essential oils formulation against *Plutella xylostella* L

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Four essential oils formulations originated from Melaleuca alternifolia and Eucalyptus were evaluated for their insecticidal effect against Plutella xylostella L (Lepidoptera: Plutellidae). This insect is the major pest of Brassica plants. The essential oil formulations used in this study are funga-t-ol; gamma-t-ol; funga-t-ol and neem oil; and gamma-t-ol and neem oil. All the essential oils examined revealed insecticidal effects and antifeedant activities against P. xylostella larvae. By using leaf dipped methods, the oils formulation reduced the larval and pupal weight. Furthermore, malformation during pupal stage was also recorded. Oviposition deterrent effects when an adult stage was exposed to treated leaves were found to be significant (P = 0.0001) compare to control. No eggs have been laid at concentration above 4.6% for all essential oils applied. The toxicity assay by looking the LC50 indicates the insecticidal potential in the essential oil of gamma-t-ol is more toxic than funga-tol when the oil applied independently. However, the presence of neem oil into funga-t-ol and gamma-t-ol increased their insecticidal activity by looking at the higher antifeedant index, the lower LC50, insect weight and insect fecundity. Therefore, these results suggest that essential oils from the studied plants may be used against insect pests in P. xylostella. In addition, it appears that neem oil addition can increase the efficacy of funga-t-ol and gamma-t-ol.

Keywords:*Funga-t-ol, Gamma-t-ol, Neem oil, Plutella xylostella, efficacy*

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Thermal tolerance, acclimation responses and voluntary running performance of meat ants (*Iridomrymex purpureus*): Implications of climate change on common species

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Understanding thermal performance and limits to activity or survival of is of fundamental importance to predicting the effects of climate change on terrestrial organisms. Here we examine several traits of physiological tolerance and voluntary running performance on an Australian meat ant (Iridomrymex purpureus), and how these traits changed with acclimation to varying temperatures in the laboratory and between summer and winter seasons. Specifically, we predict that meat ants will exhibit 1) limited rapid-cold hardening responses due to their high mobility and behavioural flexibility, 2) pronounced acclimation and seasonal responses of thermal tolerance owing to high levels of seasonal variability and predictability in their natural habitat, and 3) that voluntary performance in the field is temperature-dependent and similar to laboratory thermal performance curves and 4) that acclimation responses of running speed will be limited. We discuss our results in the context of climate change impacts on common dominant species, and the importance of local microclimate recordings.

Keywords:*Physiology, behaviour, ecology, ants*

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Does size matter? Insights from the life history traits of dengue vectors in Kolkata, India

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In mosquitoes, morphological features are often viewed as life history traits providing indication about individual and population level fitness. Fitness of mosquitoes enables predictions about prospective population build up and disease transmission potential. Using body size as surrogate of fitness, an estimation of its correlation with various life history traits of the dengue vectors Aedes aegypti and Ae. albopictus was made. Adult body size expressed in terms of wing length and dry biomass was significantly positively correlated with longevity and fecundity of both Aedes species. Larval developmental time and pupal weight were positively correlated to wing length, biomass and nutritional reserves in the adult Aedes mosquitoes. Species specific and sex specific differences were prominent for these measures and Ae. aegypti being larger in body size than Ae. albopictus. It is apparent that larval development time is key determinant of body size that in turn assures fitness of adult Aedes mosquitoes. Since dengue transmission is linked to longevity and fecundity, body size of Aedes mosquitoes is a key factor in dengue epidemiology. Aedes mosquitoes will be favoured by natural selection for bigger body size to ensure higher reproductive success and thus sustenance of a larger population; it would also increase the risk of dengue in human. Thus factors favouring the development of individual larva into bigger adults would indirectly promote dengue prevalence in a geographical area. Body size therefore is an important morphological feature that matters in dengue transmission and prevalence.

Keywords:*Aedes aegypti, Ae. albopictus, life history traits, nutritional reserve, fitness*

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Managing Potato virus Y (Potyviridae): The role of aphid (Aphididae: Hemiptera) olfaction in identifying potential crop border plants

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Potato virus Y (PVY) causes downgrading of seed potato quality leading to severe economic losses. Crop borders, consisting of virus non-host plants, can reduce the incidence of non-persistently transmitted viruses, such as PVY. Immigrating aphid vectors land in high numbers at the edge of a field. Planting a non-host plant for the virus displaces the border by acting as a virus sink. Using a plant species/cultivar preferred by aphids would considerably strengthen the effect. The aim of this study is to select a border plant from maize and wheat cultivars that is more attractive to aphids than potato. The olfactory response of the PVY vectors Rhopalosiphum padi (L.) and Macrosiphum euphorbiae (Thomas) (Hemiptera: Aphididae) to three maize, potato and wheat cultivars each (referred to as cultivars 1, 2 and 3) was evaluated using an olfactometer. Rhopalosiphum padi was attracted to odours from maize cultivar 2, potato cultivar 3 and wheat cultivar 2. Macrosiphum euphorbiae spent more time in treated arms of maize cultivar 2 and 3, and wheat cultivar 2 and 3. Analysis of plant volatile profiles by gas chromatography/mass spectrometry revealed quantitative and qualitative differences in the volatile profiles of the crop cultivars, potentially explaining the observed differences in cultivar preferences of the aphids. In regions where R. padi and M. euphorbiae are abundant maize cultivar 2 and wheat cultivar 2 may have the highest potential as crop border plants.

Keywords:*Aphid, olfaction, crop border, PVY*

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Characterization of resistant factors in faba bean (*Vicia faba* L.) cultivars against cowpea aphid (*Aphis craccivora Kosch.*) using Electrical Penetration Graph (DC-EPG)

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[1]King Saud University, Saudi Arabia, [2]King Saud University, Saudi Arabia, [3]King Saud University, Saudi Arabia

Potential resistance of five faba bean (Vicia faba L.) cultivars against cowpea aphid (Aphis craccivora Kosch.) (Homoptera:Aphidae), was characterized through colony-development study, biological study, and feeding behaviour, using Electrical Penetration Graph (DC-EPG). The last two experiments were conducted by distinguishing whole plant and detached leaf assays. Colony-development study ranked the cultivars from the highest resistance to susceptible level into Gazira 2>commercial 'Misr'>Giza 3 improved>Goff 1>Misr 1. The aphid biological study (whole plant) confirmed the highest resistant level was found in Gazira 2 among five selected faba bean cultivars, which was indicated by relatively low total numbers of progeny, shorter reproductive time, and longest total longevity, although it did not differ significantly. In feeding behaviour study, the different resistant levels among the five faba bean cultivars were not due to surface or phloem tissue factors, but rather because of the duration of stylet derailment (waveform F), in which whole plant Gazira 2 had the longest duration, however, this was not retained in the detached leaf. Principle component analysis for some selected feeding parameters showed significant difference in aphid performance between whole plant and detached leaf. The combination of colony-development and feeding behaviour studies using DC-EPG shown to be an effective way to locate the resistant factors in faba bean cultivars against cowpea aphid.

Keywords:*Faba bean, cowpea aphid, biological study, colony-development study, DC-EPG, feeding behaviour*

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Study of life history of *Hemipteroseius indicus* (Acari: Otopheidomenidae) parasitic on *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) in Kolkata, India

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Dysdercus koenigii is the key pest of cotton and several other crops of Malvaceae throughout tropical and subtropical parts of the world. India being the global second largest producer of cotton suffers from continuous retarding production due to damage to the host plants by Dysdercus koenigii. Hemipteroseius indicus was found to parasitize the gregarious pest. The life cycle of Hemipteroseius indicus on Dysdercus koenigii was studied under laboratory condition in Kolkata, India at two temperature regimes of 19 °C and 33°C. To each of the cotton seedlings with cotton balls and uninfested pests, single gravid female mite (obtained from pure culture) was released on the dorsal surface of the abdomen and a replicate of 15 such seedling were set up. After 24 hours when the eggs were found laid, the females were removed and the eggs were observed every 24 hours till they hatched and reached the adult stage. During summer the males and females have a higher longevity (male: 15.16 ± 0.60, female: 18.84 ± 0.63) and fecundity (16.25 ± 1.20) as compared to winter (♂LONG: 8.33 ± 1.20, ♀LONG: 11.13 ± 0.72 and fecundity= 0.79 ± 0.08). The difference in the duration of other stages too, at two temperatures was significant which may be an indication of differential life history modification due to thermal variation. Thus the study highlights thermal tolerance and threshold of a natural enemy which is a critical factor in the evaluation of its suitability as a biological control agent.

Keywords:*Dysdercus koenigii, cotton pest, parasitic mite, Hemipteroseius indicus, life-cycle, biological cont*

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Effects of gibberellic acid on hemolymph antioxidant enzymes of *Galleria mellonellaL.*(Lepidoptera: Pyralidae)

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The impacts of the plant growth regulator, gibberellic acid (GA3) given in diet on the activity of Glutathione S-transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) which are antioxidant enzyme in hemolymph were investigated using Galleria mellonellaL. (Lep.: Pyralidae) larvae. G. mellonella last instar larvae were reared in the laboratory at 25 ± 2 °C, 60 ± 5 % relative humidity, and a photoperiod of 12:12 h. Assays with GA3 were also carried out in the same laboratory condition. To investigate the effects of different doses of GA3 (50 to 5,000 ppm), enzyme activities were analyzed using spectrophotometry and enzyme-linked immunosorbent assay (ELISA) methods. Exposure of G. mellonella to a diet containing GA3 resulted in a remarkable increase in the activity of GST at 200 ppm with respect to control and other doses. The activity of GST increased at 200 and 500 ppm because of inhibiting effect of GA3. The activity of CAT in the hemolymph of last instar larvae increased significantly at higher doses > 100 ppm compared with untreated larvae. This trend in increase was not dose-wise except for the significant increase at 5,000 ppm when compared to that of untreated and all treated groups. Consequently, our results showed that GA3 is effective on the antioxidant defense system of insects as a source of free radical. Therefore, we suggest that the increase or decrease in the activity of GST, SOD and CAT in larvae may indicate a physiological adaptability to compensate for GA3-induced stress.

Keywords:*Galleria mellonella, Gibberellic Acid, Catalase, Glutathione S-transferase, Superoxide Dismutase.*

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Novel interactions of a cypovirus in the *Heliothis virescens* and *Campoletis sonorensis* host-parasitoid system.

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Some viruses, notably the ascoviruses, are reliant upon parasitoid wasps for horizontal transmission between their lepidopteran hosts. Other viruses, the polydnaviruses, are obligate symbionts of parasitoid wasps, existing in highly commensal and evolved relationships with their parasitoid hosts. Among the viruses associated with parasitoid wasps, polydnaviruses are considered beneficial to the wasp and are essential in the parasitism of lepidopteran hosts. In contrast, the impact of ascoviruses within the wasp-host system can be detrimental to both the wasp and host. There are many viruses, however, associated with parasitoid wasps or the lepidopteran host, and little is known about their impact on the host-parasitoid system. We have recently discovered two genetic variants of a dsRNA Cypovirus (CPV) in laboratory colonies of the parasitoid wasp, Campoletis sonorensis and in the wasp's conventional host, Heliothis virescens larvae. CPVs typically cause non-lethal effects in insects and are similar, but unrelated, to Baculoviruses as the virions are occluded and protected by a polyhedral matrix. We characterized and sequenced the CPV variants and discovered a mutation in the polyhedrin-encoding segment. We then examined the unique pathologies and biological effects of these variants in our C. sonorensis and H. virescens colonies. CPVs are common insect colony pathogens, yet little is known about their effects on a complex biological system. We describe here a novel virus which appears to interact with a host-parasitoid system in a fundamentally new and intriguing way.

Keywords:*polydnavirus, cypovirus, parasitoid, CPV, polyhedra*

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P7

The preservation of extracted insects from Lower Eocene Indian amber

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Amber preserves a diverse array of organisms from different habitats living in the amber-producing forest. The detailed analysis and comparison of amber inclusions reveals major aspects of the evolutionary history of these organisms. Indian Cambay amber was deposited about 50-52 million years ago during the Early Eocene Climatic Optimum (EECO), at a time approaching the Indian subcontinent's collision with Asia and is now located in western Gujarat, India. The fossil resin from the Vastan and Tadkeshwar lignite mines has preserved a highly diverse flora and fauna, including 22 orders and more than 60 families of arthropods so far. The source of the fossil resin were trees of the Family Dipterocarpacea, producing a class II dammar resin. Usually fossil arthropods are preserved in amber only as hollow spaces lined with a thin layer of diagenetically altered or decomposed cuticle. In sharp contrast, arthropods in Indian amber are often completely preserved including the cuticle as well as internal tissues of the embedded organisms, such as muscle tissue or the tracheal system. The poor polymerization and crosslinking of the amber, combined with the exceptionally preserved arthropods allow the complete extraction of the arthropod inclusions with different chemicals. Afterwards the isolated arthropods can be examined with scanning electron microscopy, enabling submicron resolution of morphological details, but also histological analyses, examinations with transmission electron microscopy as well as histochemical and other molecular methods are applicable.

Keywords:*amber, preservation of arthropods*

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The butterfly diversity of Huoditang, Qinling Mountain in China

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The diversity of butterflies in Huoditang area of in Qinling Mountain were investigated to test the effect of altitude on this diversity. The results showed that a total of 432 butterflies, belonging to 76 species, 42 genus and 7 families was collected. Based on the numbers of individuals and species, Nymphalidae is the dominant population in this area while Libytheidae is the rare population. As the increase of altitude, the individuals and species of butterflies first increased and then decreased. The richness index E and diversity index H decreased while the Evenness index J and dominance index C did not change obviously. The similarity indices in different altitude series were significantly different, which meant that this diversity had relations with the altitude and climate. The number of species and individuals, diversity index of butterflies were highest when the altitude is between 1500and 1600m. This area maintained highest butterfly diversity, so it should be well protected.

Keywords:*Qinling Mountain,altitude,diversity*

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Rapid molecular identification of Encyrtid and Aphelinid parasitoids of soft scales (Hemiptera: Coccidae) in Californian citrus orchards using taxon-specific multiplex PCR

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Citricola scale, Coccus pseudomagnoliarum Kuwana (Hemiptera: Coccidae), is a serious pest of citrus in California's San Joaquin Valley, but not in southern California where it is suppressed by a complex of Metaphycus spp. Mercet (Hymenoptera: Encyrtidae). This has created interest in using these (and other Metaphycus) species for biological control in the San Joaquin Valley. A critical step in assessing an organism's potential for biological control is the ability to accurately identify it. For Metaphycus spp., this currently requires slide mounted adult specimens and expert taxonomic knowledge. We present a simple, quick and accurate method to identify any life stage of the 10 major parasitoids of soft scales in California citrus, based on amplification of ribosomal DNA, using the polymerase chain reaction (PCR). This includes three multiplex-PCR protocols each of which amplify products of taxon-specific sizes, allowing direct diagnosis of taxa accommodated by the PCR. The method does not demand a highly skilled operator, requiring knowledge of only a few basic molecular techniques, and reduces identification time and cost to a fraction of that of existing methods.

Keywords:*Hymenoptera, Metaphycus, Coccophagus, Microterys, rDNA, citricola scale*

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The action mode of the nitroconjugated neonicotinoids with -NO2 or -CN group in cis-configuration

Haibo Bao[1], Qian Cheng[2], Jing Yao[3], Haiyan Lu[4], Zewen Liu[5]

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Neonicotinoid insecticides, such as imidacloprid, are selective agonists of the insect nicotinic acetylcholine receptors (nAChRs) with -NO2 or -CN group in trans-configuration. Insecticide resistance to neonicotinoids was reported in several insect species. In order to find out the new neonicotinoids without the cross-resistance, a series of nitroconjugated neonicotinoids with -NO2 or -CN group in cis-configuration were synthesized and proved to possess excellent insecticidal activity, but without the significant cross-resistance with the commercial neonicotinoids. The representative members, IPPA152004 and IPPA152201, were chosen to perform toxicity and pharmacology studies. IPPA152004 still acted on insect nAChRs, but discriminated between the high and low affinity imidacloprid binding sites in the brown planthopper, Nilaparvata lugens. In Xenopus oocytes co-injected with cRNA encoding Nlα1 and rat β2 subunits, the previously identified Y151S mutation in Nlα1 showed significant effects on the agonist potency of IPPA152004, which was less than that on imidacloprid. IPPA152201 showed a comparable toxicity with imidacloprid against N. lugens in a susceptible strain and had no significant cross-resistance in an imidacloprid resistant strain. IPPA152201 showed good efficacies on the isolated cockroach neurons and hybrid receptors Nlα1/β2 in Xenopus oocytes, whose responses were inhibited by MLA and DHβE. Compared to the wildtype Nlα1/β2, Y151S mutation reduced Imax of IPPA152201 and increased EC50, however, which is much smaller than that on imidacloprid.

Keywords:*Neonicotinoid, cis-configuration, insecticidal activity, cross-resistance*

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Laboratory production of green lacewings, *Plesiochrysa ramburi* (Schneider)(Neuroptera: Chrysopidae) for augmentative biocontrol ofthe mealybug complex in cassava plantations

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The green lacewings, Plesiochrysa ramburi (Schneider) (Neuroptera: Chrysopidae), is widespread in cassava plantations in Thailand, but at low number and for the catastrophic outbreaks of the mealybug complex in cassava in 2009, mass-production of the predators is imperative. The development of the mass-production of the green lacewings aimed to reduce cost and increase efficiency in the production of the predators. Primarily, mass-production of the preys was assessed in four densities 50, 100, 150 and 200 individuals of the mealybug, Phenacoccus manihoti, Matile-ferrero (Hemiptera: Pseudococcidae)/pumpkin, in dark room with 25±1˚C and 50±5 % RH, in four weeks duration. Each pumpkin was weighted approximate 1 kg. Number of the mealybug increased significantly ($P<0.05$), with each initial density. At the density 200 it gave 33,394.70±7,483.062 mealybugs/pumpkin, followed by 150, 100, and 50 /pumpkin, averaging 24,387.50±7,320.604, 16,479.80±3,002.616, and 10,024.93±2,161.204 /pumpkin, respectively. Later the larvae of the predators in four densities 50, 100, 150 and 200 were placed on the pumpkins which had different number of the mealybugs, obtained from the previous experiment. Among the test treatments combination of 100 mealybugs/pumpkin at the initial of experiment, i.e. averaged 16,479.80 preys/pumpkin and 100 larvae of the green lacewings/pumpkin provided the survival of larvae to adults 60.08%, yielding significant ($P<0.05$) the best combination. Ultimately from October 2010-September 2011, 18.41 million eggs of the green lacewings, with a production cost 20 US$ per one thousand eggs, were accomplished and 12.54 million larvae were disseminated for augmentative release in cassava plantations with the mealybug outbreaks.

Keywords:*green lacewing, Plesiochrysa ramburi, Phenacoccus manihoti, augmentative release, mass-production*

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Biology of *Bracon hebetori* Say (Hymenoptera: Braconidae) and its potential in population suppression of the black-headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae)

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Bracon hebetor Say is an ectoparasitoid of the black-headed caterpillar, Opisina arenosella Walker. Because of the O. arenosella devastating outbreak in coconut plantations in Prachuap Khiri Khan province, Thailand since December 2010, biological information of the parasitoid on its insect host has to be urgently needed. The egg stage of B. hebetor was 1 day. and it had two larval instars with an average 2 days. The pupal period took 5.28±0.51 days. Female and male sagittal measurements averaged 2.09±0.26 and 2.08±0.18 mm, respectively. Transversal measurements averaged 0.57±0.07 and 0.56±0.06 mm for female and male, respectively. Female lived longer than male, averaging 8.68±5.29 and 7.59±3.37 days, respectively. Female laid averaged 166.88±18.53 eggs/female for its entire life. Additional comparative field study on the control tendency of B. hebetor as a biocontrol agent for O. arenosella was conducted in coconut plantations in the outbreak areas from January-July 2011. Eight coconut plantation plots, 1,600 sq.m each, were employed to observe the efficiency of the parasitoid in suppressing the naturally occurring O. arenosella populations. Two treatments, unreleased and released adults of B. hebetor with a rate of 200/month/plot were administered. Each treatment replicated 4 times. Sampling of the number of O. arenosella before and after release the parasitoids. Four trees/plot and four pinnate leaves/tree were randomly sampled on each sampling date. Results indicated that considerable control potential, within six months, averaged 21.22, 66.46, 35.26, 47.05, 64.96 and 71.86%, was recorded, respectively. B. hebetor tends to be a potential parasitoid species in controlling O. arenosella.

Keywords:*Biology, Bracon hebetor Say, Opisina arenosella Walker, population suppression*

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RNAi target validation of juvenile hormone related genes for developing novel insect growth regulators in the brown planthopper, *Nilaparvata lugens*

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The brown planthopper (BPH), Nilaparvata lugens, is one of the most destructive pests of rice in Japan. Thanks to the use of imidachloprid, the damage by BPH once became marginal in Japan. However, the outbreaks of BPH have been observed again after 2005 due to the migration of BPH that has developed resistance to the insecticide from China and South Asia. To solve this problem, development of new insect growth regulators (IGRs) which have different mode of actions is expected. Using the genome and transcriptome data of BPH, we have identified genes and/or cDNAs involved in the biosynthesis or signal transduction of juvenile hormone (JH), and evaluated their property for the target of new IGRs using RNAi techniques. By injecting respective dsRNA to the day 0 third instar nymphs, CYP15A1, a JH biosynthesis enzyme gene encoding a methylfarnesoate epoxidase, induced precocious adults at the fifth instar stage. While RNAi of JH acid methyltransferase (JHAMT) did not apparently affect their development. Intriguingly, in the red flour beetle, Tribolium castaneum, JHAMT RNAi induced precocious metamorphosis, but CYP15A1 RNAi didn't, suggesting the difference of importance of these genes in JH biosynthesis between two species. RNAi of Kruppel homolog 1 (Kr-h1), an early JH-inducible transcription factor, induced precocious metamorphosisis like CYP15A1 RNAi. While RNAi of the JH receptor, methoprene tolerant (Met) did not induce precocious metamorphosis but resulted in high mortality during nymphal stages. These results suggest that CYP15A1, Met, and Kr-h1 are plausible molecular targets for new IGRs counteracting the resistant BPH.

Keywords:*Insect growth regulators, Juvenile hormone, RNAi, Nilaparvata lugens, Insecticide resistance*

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Spider mite response in citrus rootstocks is mediated by the oxylipin pathway

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Citrus rootstock selection has been traditionally based on its resistance to pathogens and tolerance to abiotic stress. However, the resistance to insects has been consistently neglected. Recently, it has been shown that the citrus rootstock can influence the life history of the phytophagous spider mite Tetranychus urticae. To compare the usefulness of pathogen resistance-based rootstock selection with arthropod tolerance/resistance we have performed comparative experiments of spider mite performance on Carrizo Citrange (Citrus Tristeza Virus, CTV, tolerant), Cleopatra mandarin (CTV and salt tolerant) and Sour orange (CTV sensitive and limestone tolerant). Sour orange showed reduced leaf damage symptoms and supported lower mite populations. Taking together the genetic analysis of defense pathways and the phenotype, results suggest a relevant role of the oxylipin pathway in the plant's defense against T. urticae. Nevertheless the SA-related responses cannot be excluded, since the most resistant rootstock, Sour orange, still displayed elevated levels of this hormone and PR5 marker gene expression upon spider mite infestation. Because the LOX inhibitor Phenidone totally abolished the enhanced response of Sour orange, oxylipins are probably essential signals controlling the orange tree defense responses against arthropod infestations.

Keywords:*Tetranychus urticae, defense pathways, Sour orange, Carrizo citrange, Cleopatra mandarin*

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Claiming the crown by primary queen and king after nuptial flight in orphaned conspecific colony

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Of social insects, termites show the most complex and sophisticated reproductive system, which resulted in diverse colony genetic structures and behavior plasticity. A colony can be headed by a primary pair or various forms of supplementary reproductives. We report here a new reproduction in two subterranean termite species, Reticulitermes flavipes and R. malletie (Isoptera: Rhinotermitidae). In this study, we introduced a pair of queen and king after nuptial flight to orphaned worker groups from their natal colony, an alien conspecific colony, or an interspecific colony, to mimic possible colony infilation by newly swarmed dealates in the field. The pairs introduced into orphaned interspecific worker groups were killed immediately. The pairs introduced to intraspecific worker groups experienced moderate agonism. However, 100% of the pairs (n=6) survived in natal worker groups and 20% of the pairs (n=12) survived in conspecific worker groups. The survivor pairs of R. malletei were further assessed for survivorship and capacity of reproduction over a 7-week period. Five of the six pairs had both or one partner died within 25 d and neotenic reproductive were observed in orphaned worker groups. One pair survived and produced eggs at 23 d and the eggs hatched at 34 d. In all the groups, workers had a survivorship >75%. This study presents the first ethological evidence of the adoption of primary reproductive re-entering their natal or conspecific colony after a dispersal flight and show that eusociality can generate diverse reproductive systems.

Keywords:*subterranean termites, Reproduction strategy, queen, king adoption*

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Effect of pollen quality on biological control of *Tetranychus urticae* in citrus

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Cover crops can modulate phytoseiid communities through provision of non-prey food sources as pollen. Clementine trees associated with a mono-specific cover crop of Festuca arundinacea had a more diverse and efficient phytoseiid community than those associated with a multifloral cover. Therefore, the former had better regulation of Tetranychus urticae populations than the latter. Pollen availability and quality could be behind this result because a multifloral cover offers longer provision of higher quality pollen relative to F. arundinacea. Therefore, populations of the superior intraguild predator and less efficient generalist pollen feeder Euseius stipulatus could easily increase in multifloral covers and then interfere with the biological control of T. urticae exerted by specific phytoseiid predators (Neoseiulus californicus and Phytoseiulus persimilis). To determine whether pollen quality could be behind these results, the effect of the provision of two types of pollen (F. arundinacea and Carpobrotus edulis) on the efficacy of two different life-style phytoseiid species (E. stipulatus and N. californicus) to regulate T. urticae populations has been studied under semi-field conditions. Results suggest that pollen provision does not enhance the ability of these phytoseiids to reduce T. urticae populations. However, C. edulis pollen resulted in explosive increases of E. stipulatus numbers that did not occur with F. arundinacea pollen. Therefore, poor quality pollen may prevent pollen feeders from reaching high numbers in the field. This effect could benefit phytoseiid species suffering intraguild predation by generalist pollen feeders, as in the case of E. stipulatus, the predominant phytoseiid species in Spanish citrus orchards.

Keywords:*Cover crop, Conservation biological control, Phytoseiidae, Tetranychidae*

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Effect of nutrient and pre-infested Brinjal plants, *Solanum melongena* on the population and interaction between whitefly and competitor pest in a multitrophic System

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This study focused on the insect-pest composition on the underside of leaves of whitefly and aphid pre-infested brinjal plants fertilized with three levels of nutrients, N1 (50 ppm=0.05g/L), N2 (150 ppm=0.15g/L) and N3 (300 ppm=0.3g/L). The common insects-pests found on brinjals leaves were mainly sucking insects, for instance, whitefly (Bemisia tabaci), aphid (Myzus persicae Sulzer) and thrips (Thrips palmi). Based on the result from two cropping periods, once treatments applied on brinjal plants; nutrient levels that commonly influenced plant performance and pre-infestation by pests which scientifically known triggered chemical defense earlier, had significantly influenced the population abundance of whitefly, aphid, thrips, mite and spider mite. Other than treatments' effects on host plants, the population of whitefly evidently was regulated by other competitor pests naturally occurring in the field. High diversity of plant pests could modify the main pest population (whitefly) by either increasing or reducing and mediating the pest effect. When aphid co-exist to develop and compete with whitefly on the brinjal leaves, there was significant mutual relationship occurred under agro-ecological system particularly under rainshelter structure. Variations in population abundances and interactions of insect-pest community in a multitrophic system generate ideal opportunity to understand the community ecology more accurately.

Keywords:*Solanum melongena, multitrophic system, Bemisia tabaci, Myzus persicae, Thrips palmi*

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Effect of imidacloprid and fipronil pesticide application on *Sympetrum infuscatum* (libellulidae: Odonata) larvae and adults

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The effect of imidacloprid and fipronil on Sympetrum infuscatum larvae and adults during the rice cultivation period was monitored using an experimental micro paddy lysimeter (MPL) system. Twenty-two hatched larvae were laid on the soil surface of each MPL. MPLs were treated with imidacloprid, fipronil, and the control MPL was left untreated. The pesticide concentration, S. infuscatum larval and adult populations, and larval emergence time were monitored in each MPL. The maximum imidacloprid and fipronil concentration in paddy water was 52.8 ?g/l at 1 day, and 1.3 ? g/l at 6 h, respectively, after the pesticide application. Both pesticides dissipated quickly in paddy water, with half-lives of 8.8 days and 5.4 days for imidacloprid and fipronil, respectively. The absence of S. infuscatum larvae and exuviae in the fipronil-treated MPL was remarkable. The larval survival percentage decreased to 63.6 ± 18.2%, 15.2 ± 2.6%, and 0% in the control, imidacloprid-treated, and fipronil-treated MPLs, respectively, by 9 days after transplantation. Emergence in the imidacloprid-treated MPL was also significantly lower than that in the control MPL. The observed decrease in the abundances of Sympetrum infuscatum larvae and adults in MPLs seems to be both directly and indirectly associated with nursery-box application of fipronil and imidacloprid.

Keywords:*microcosm, micro paddy lysimeter, Sympetrum infuscatum, imidacloprid, fipronil*

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Embryogenesis of tracheal gills in *Dineutus mellyi* (Coleoptera, Gyrinidae): Their subcoxal origin

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The larvae of the Gyrinidae are unique in having 10 pairs of tracheal gills; eight pairs of them in the first eight abdominal segments, and two pairs in the ninth segment. In the present study, the formative process of the tracheal gills in the embryo of a whirligig beetle, Dineutus mellyi, was described from observations based on light and scanning electron microscopy. A pair of lateral tracheal gills (LTGs) of the first abdominal segment originates forms appendage-like projections arising at the lateral side of pleuropodia, and the LTGs of the second to ninth abdominal segments are arranged in a row with that of the first segment. Therefore, the LTGs are structures with serial homology. The paired dorsal tracheal gills (DTGs) of the ninth abdominal segment are formed on the regions just latero-dorsal to the LTGs of this segment. Regarding the pleuropodia as the structures being homologous with thoracic legs, neither the LTGs nor DTGs are homologous with thoracic legs, but originate in the more lateral region corresponding to the future pleura of the thoracic segments. Four terminal hooks at the end of the last abdominal segment originate from two pairs of swellings on the posterior end of the embryonic eleventh abdominal segment. In conclusion, our observations reveal that both the LTGs and DTGs originate in future pleural regions. Recent embryological studies have demonstrated the subcoxal origin of insect pleura. Therefore, assuming the subcoxal nature of pleura, the LTGs and DTGs might correspond to subcoxal exites.

Keywords:*Gyrinidae, Dineutus, embryo, tracheal gill, subcoxa, pleuropodium, exite*

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Quarantine Insect Species in the Russian Federation

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Lately, the number of countries exporting plant products into Russia has significantly increased. This has enhanced the risk of quarantine pests being introduced into the country which may have a serious economic impact. The All-Russian Plant Quarantine Center, as well as its 25 branches throughout the country, is responsible for handling pest threats. There are 39 quarantine insect species included into Russia's List of Quarantine Species. Seventeen of these species are absent in Russia and twenty two have limited distribution. The most dangerous of the introduced species are Frankliniella occidentalis (Pergande, 1895) (Thysanoptera: Thripidae), Bemisia tabaci (Gennadius, 1889) (Homoptera: Aleyrodidae) and Phthorimaea operculella (Zeller, 1873) (Lepidoptera: Gelechiidae). Currently, the range of these species is rapidly widening. One of the recent quarantine species worth mentioning is Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae). It was introduced into Anapa and Novorossiysk in 1994. The pest outbreak is currently expanding. In August 2011, a dangerous quarantine pest, Diabrotica virgifera virgifera Le Conte, 1868 (Coleoptera: Chrysomelidae), was detected for the first time in Rostov region. The results of laboratory tests show that F. occidentalis, Pseudaulacaspis pentagona (Targioni-Tozzetti, 1886) (Homoptera: Diaspididae) and Grapholita molesta (Busck, 1916) (Lepidoptera: Tortricidae) are the most commonly intercepted quarantine pests in imported plant products. There are also single cases of other quarantine pests being intercepted.

Keywords:*Plant Quarantine, insects, Russian Federation*

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Remipedia ovarian characters support affinity of Crustacea and Hexapoda

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Remipedia are rare, marine crustaceans living in anchialine caves. Their bodies combine both primitive and advanced characters and therefore the phylogenetic position of Remipedia within Crustacea remains controversial. The features of the ovary organization and ultrastructural aspects of oogenesis have been proven useful in resolving phylogenetic relationships in various arthropod groups. The structure of the female gonads in Remipedia is largely unknown. Therefore, we have examined overall morphology and ultrastructural details of the ovary in a remipede, Godzilliognomus frondosus focusing on characters relevant to phylogenetic reconstructions. The ovaries of G. frondosus are located in the anterior part of the body and are composed of a single anterior proliferative zone (the germarium), and paired posterior ovarian tubes (the vitellarium). During early oogenesis, somatic follicular cells envelope each oocyte creating distinct ovarian follicles. Throughout oogenesis, the follicular cells form a continuous but unpolarized layer on the surface of the oocyte. Because the follicles develop within the lumen of the ovarian tubes, the remipede ovaries can be classified as endogenous. Our results demonstrate that Remipedia share significant similarities in the ovary organization with Cephalocarida but also with the basal hexapod group, the Entognatha, including the subdivision of the ovary into a well defined anterior germarium and an elongated posterior vitellarium, localization of the germaria within the trunk or head region of the body, and simple, morphologically unpolarized follicular cells. Our findings give strong support for the Pancrustacea hypothesis. This work was supported by grant 2011/01/B/NZ4/00595 from the National Science Centre.

Keywords:*ovary structure, oogenesis, Crustacean evolution, Entognatha, Pancrustacea*

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Progress on chemical utilization of cochineal insect

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Cochineal, the insect originated from Latin America, has been developed since its introduction to China. Its red dye, wax and other chemical substances were investigated. Most of the concerns were focused on carminic acid or carmine, the anthroquinone structural and the main component of cochineal dye. The comprehensive studies on its preparation were taken across extraction, separation, purification, drying and instrumental analysis. It was seldom used today with the traditional way of soaking directly in water for the extraction. Other physical aids such as microwave and ultrasonic wave were combined for its acceleration. To isolate carmine from protein, sugar and other impurities, adsorptive resins and enzymes were often selected for a highly purified product. Aimed to the prevention of oxidation, the extracted liquid was treated immediately by vacuum freeze drying, spray drying or other drying operations. And the dried powder could be analysed by spectrophotometry or high performance liquid chromatography (HPLC), according to different need of carmine determination with high precise or short time. Coccerin was also a natural insect wax but was not developed industrially. Its fine characteristics of whiteness, melting point, acid value and saponification value were similar to other natural bio-wax. Chitin from some cochineal species was available by usual extraction and separation, although within a low yield. It was suggested that the abundant protein and other attached constituents in the insect should be fully utilized in the future. An integrated industry of cochineal was expected to be formed in China soon.

Keywords:*cochineal, extract, dye, wax*

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Diurnal locomotion and feeding behaviors of rice-ear bugs measured by electrical penetration graph and video camera

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"Pecky rice" is one of the most serious problems facing rice cultivation in Japan, causing enormous economic losses. Pecky rice is the name given to rice grains fed on by stink bugs called rice-ear bugs. Two mirid bugs, Trigonotylus caelestialium and Stenotus rubrovittatus, are among the most important rice-ear bug species. Although these 2 mirids are well studied in Japan, their daily behaviors are poorly understood. In this study, we investigated the diurnal locomotion and feeding behaviors of T. caelestialium adults by using electrical penetration graph (EPG) and video camera recordings. According to EPG recordings, feeding behavior was more common in photophase than in scotophase. The diurnal rhythms of feeding behavior were similar between mated and non-mated bugs but not between males and females, suggesting that the diurnal feeding behavior of this bug was influenced by mating experience as opposed to gender. Locomotion activity measured by a video camera was higher in photophase than in scotophase. However, the diurnal rhythms of locomotion activity were similar between males and females but not between mated and non-mated bugs, suggesting that diurnal locomotion activity was influenced by gender instead of mating experience. It is believed that this bug is active both during the day and at night, although it is more active at daytime. This study indicates that T. caelestialium is a noct-diurnal insect. In this presentation, the daily behavior of S. rubrovittatus will also be discussed.

Keywords:*Pecky rice, Trigonotylus caelestialium, Stenotus rubrovittatus, electrical penetration graph, the diurnal rhythms, noct-diurnal insect*

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Feeding and ovipositional behaviors of *Liriomyza huidobrensis* under lights of different wavelength

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Liriomyza huidobrensis is a highly polyphagous leafminer and causes serious damage to various crops. This insect is difficult to control with insecticide sprays because its larvae develop and live within plant leaves, where they are protected from the sprays. This fly is a diurnal insect, and adults puncture plant leaves for feeding and oviposition during the day. However, the wavelengths of light that regulate their ovipositional and feeding behaviors are unknown. In this study, we investigated the feeding and ovipositional behaviors of L. huidobrensis adults under LED light of various wavelengths (355, 375, 405, 470, 530, 590, 660, and 735 nm) and in the dark and under a photoregimen of 16L:8D as controls. Flies under 355?590-nm light actively laid eggs and fed on plant leaves, as did the 16L:8D control. Feeding and ovipositional activities under 735-nm light were similar to those in the dark. It was believed that the flies recognized 735-nm light as a dark condition because they cannot sense a longer wavelength than visible red light. Although feeding and ovipositional activities under 660-nm light were lower than those under 355?590-nm light and in the 16L:8D control, the activities were higher than those in the dark and under 735-nm light. It was believed that the flies could slightly sense 660-nm LED light because it has a shorter wavelength than 660 nm. This study reveals that L. huidobrensis punctures leaves for feeding and oviposition if there are any wavelengths of light they can sense.

Keywords:*Liriomyza huidobrensis, various wavelengths, feeding and ovipositional behaviors*

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Application of microsatellite markers for strain identification in sterile insect techniques: *Bactrocera dorsalis* (Diptera: Tephritidae)

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Bactrocera dorsalis (Hendel) or oriental fruit fly is one of the most destructive agricultural pests worldwide, causing a loss of fruit yield and constraints in the international market. In pest control programmes, sterile insect technique (SIT) involves field release of large number of mass-reared and sterile insects, resulting in successful suppression or eradication of a fruit fly population through infertile mating. Evaluation of SIT effectiveness requires efficient marking systems for distinguishing the released flies in monitoring traps. Accordingly, one SIT advancement concerns strain improvement. This study genetically characterized 11 microsatellite DNA markers in a mass-reared strain. Many of these markers were used for strain identification in field monitoring traps. The mass-reared strain was also validated in a pilot integrated pest management programme using male-only SIT in a fruit orchard, resulting in the suppression of the fruit fly population.

Keywords:*oriental fruit fly, Bactrocera dorsalis, microsatellites, sterile insect technique*

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Structure of the female reproductive system in viviparous earwigs

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The vast majority of earwigs are oviparous, but in representatives of two parasitic taxa (Arixeniina and Hemimerina) the viviparity was reported. It probably represents the adaptation for parasitism, increasing the chances of nymphs to find a proper host. Here we present the analysis of female reproductive system in viviparous earwig Arixenia esau. This species has paired ovaries that are composed of 3 ovarioles attached to the lateral oviduct. The oviduct is strongly dilated and contains developing embryos, and therefore can be regarded as the uterus. The uterus wall is thick and deeply folded. The TEM analyses showed that in the individual fold, two easily recognizable layers can be distinguished. The outer zone is composed of muscle fibers, between which numerous tracheal tubes are located. The inner layer is composed of a monolayer of epithelial cells lying on a well developed basal lamina. It should be added that the basal lamina also contains tracheal branches. The epithelial cells are elongated and are of the characteristic shape. In their basal regions they are significantly folded and filled with numerous mitochondria and elements of RER. In the middle part of the epithelial cells the nuclei are located. Above the nuclei, numerous vacuoles are present. The most apical parts of these cells are equipped with microvilli that protrude into the uterus lumen. Such a unique structure of epithelial cells suggests their role in the nourishment of the embryos developing inside the uterus. The study was funded by grant 2011/01/D/NZ4/00137 from the National Science Center.

Keywords:*viviparity, uterus, Arixenia, Dermaptera*

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Oogenesis in a parasitic earwig *Arixenia esau*

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The ovaries of Arixenia esau are meroistic-polytrophic and composed of 3 short ovarioles that consist of a terminal filament, germarium and vitellarium. The ovarioles are attached to a strongly dilated lateral oviduct. In the germarium the female germline cysts are located. They are always composed of two cells only: the pro-oocyte and pro-nurse cells. The nuclei of the pro-nurse cells are large, electron-dense and contain several active nucleoli, in contrast to electron-transparent oocyte nuclei. We show that in the cytoplasm of both cells of the cyst - apart from ribosomes, mitochondria and elements of RER ? numerous small polymorphic crystalline structure are located. It should be noted as well, that both cells contain in their cytoplasm the Balbiani body (Bb) composed of numerous mitochondria. In the vitellarium, one ovarian follicle is located. It is composed of a growing oocyte and a accompanying nurse cell. The oocyte is surrounded by two-layered egg envelope and a single layer of follicular cells. On anterior pole of the oocyte, the egg envelope is characteristically folded and forms peculiar specialization with visible channels inside. The follicular cells are flattened and comprise relatively big nuclei with deeply folded nuclear envelope and several nucleoli inside. It should be noted that more numerous and more prominent invaginations of the follicular cell nuclei are located on the side facing the oocyte. Thick ovariolar sheath composed of muscle fibers and plenty of trachea surrounds the whole ovariole. The study was funded by grant 2011/01/D/NZ4/00137 from the National Science Center

Keywords:*Oogenesis, germline cysts, Arixenia, Dermaptera*

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Study on the correlation between the diversity of owlfly wing shape and geographical distribution

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Owlflies (Ascalaphidae) belong to Neuroptera. More than 420 species of this family were recorded all over the world. Owlflies look like Dragonfly in having developed wings and being good at fly. Ascalaphidae has three subfamilies, Albardiinae, Haplogleniinae and Ascalaphinae. Haplogleniinae distributes mainly in America and Africa. Based on the morphological study, we observed that the wing shapes of Ascalaphidae present rich diversity, especially in different geographical areas. To clarify the correlation between the diversity of wing shapes and geographical distribution, we selected 10 species from American and other 10 species from Africa, all species belong to the subfamily Haplogleniinae. The image of the forewing and the hindwing of those 20 species were obtained. The outline characters were described as closed curve by elliptic Fourier analysis (EFA). The differences of quantitative information on wing shape between the 20 species were tested with multivariate statistical analysis. The studying results show that the 20 species of Haplogleniinae present two groups based on the quantitative characters of wing outline, one group includes all 10 American species, and the other includes all 10 African species. This result indicates that the correlation between the wing shape diversity and geographical distribution is significant in the subfamily Haplogleniinae. The wing shape differences between America species and Africa species is more distinct than the wing shape differences between the species in the same continent. This conclusion needs further study with more samples.

Keywords:*Ascalaphidae, wing shape diversity, geographical distribution, Elliptic Fourier Analysis*

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Exploration and characterization of genes involved in the diterpene synthetic pathway for defensive secretion in nasute termite soldiers

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Soldiers of the nasute termite Nasutitermes takasagoensis discharge defensive substance from frontal gland in their heads. It has been revealed that the major component of the defensive secretion is cembrene-derived polycyclic diterpene in Nasutitermes, but the synthetic pathway and the genes involved are largely unknown. To explore the genes involved in the synthesis of secretion, transcripts extracted from the cells of the frontal gland were sequenced by 454 pyrosequencing. A total of 50,290 clean sequences were assembled into 1,189 contigs that were grouped in 774 genes (isogroups). Based on sequence similarity with known proteins of other organisms, we identified four genes associated with diterpene-synthesis: 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) synthase (HMGS), HMG-CoA reductase (HMGR), farnesyl diphosphate synthase and geranylgeranyl diphosphate synthases. Assuming that the site of the defensive terpenoid synthesis strongly activates the mevalonate pathway that produces a precursor of terpenoids, two enzymes involved in the mevalonate pathway (HMGS and HMGR) were examined. Quantitative RT-PCR confirmed significantly higher expression of the HMGS and HMGR in the heads of soldiers. We then divided the head into three parts for quantitative RT-PCR and found that the expression level of HMGS and HMGR was significantly higher in a part containing the class 1 secretory cells of frontal gland. Overall, the results suggest that the mevalonate pathway of the diterpene synthesis takes place in the class 1 cells around the frontal-gland reservoir.

Keywords:*Nasutitermes takasagoensis, mevalonate pathway*

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Evaluation of modified trunk injection method for controlling *Quadrastichus erythrinae* in Taiwan

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The gall wasp, Quadrastichus erythrinae, is an invasive alien species causing severe damage to plants of genus Erythrina in Taiwan. The Q. erythrinae spends entire larva stage in its tissues, and this characteristic makes it more difficult to kill with traditional chemical control methods. At present, trunk injection is considered as one of the most effective methods for controlling Q. erythrinae. However, the effectiveness and persistence of the insecticide applied by trunk injection on native heritage trees is still unavailable. In order to quantify the dose of injection into a tree, finding out the correlation between tree diameters and injection dose should be known first. The emergence rate of Q. erythrinae shows significant difference between imidacloprid treated group and untreated one, and no difference among those trees with diameter larger than 20 cm. Monitoring results show that damage stages drop down significantly in one month after injection. HPLC analysis shows that imidacloprid can transport to the upper layer of coral tree and reach lethal dosage in 3 weeks. Not only the evaluation of trunk injection, the methods applied in this study also provides a useful reference to control other galling phytophagous pests.

Keywords:*Quadrastichus erythrinae, trunk injection, imidacloprid*

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Vitellogenin cDNA Gene of Giant Water Bug (*Lethocerus indicus*)

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[1]Kasetsart University, Thailand, [2]Kasetsart University, Thailand

Giant water bug; Lethocerus indicus, the native species of Southeast Asia, is one of the largest insects in the order Hemiptera, suborder Heteroptera. Here, vitellogenin (Livg) cDNA from L. indicus was cloned and sequenced. The complete full length Livg cDNA was 6048 bp long with the 5667 bp open reading frame (ORF) which encoded 1888 amino acid residues. The deduced LiVG protein contained the 18 amino acid signal peptide and several conserved motifs found in insect VGs. The LiVG amino acid sequence showed 92% identity to that of L. deyrollei and 30 - 49 % to those of other Hemipteran insect VGs available in the NCBI database. Phylogenetic tree established with vitellogenin proteins from some animals could group them as well as the classical taxonomy.

Keywords:*giant water bug, Lethocerus indicus, vitellogenin*

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Uffda! Selling biological control to farmers in the Upper Midwest of the USA

Kelley Tilmon
South Dakota State University, U.S.A.

Importation biological control is an effective strategy for the management of introduced pests. Releases of biological control organisms such as parasitoids help these agents to become established, but these releases also provide an opportunity for outreach and education about biological control for farmers and the public. In this poster we present some lessons learned about effective biological control Extension education from our outreach efforts surrounding the release of Binodoxys communis (a parasitoid of the soybean aphid, Aphis glycines) in the upper Midwest of the USA.

Keywords:*biological contro, Extension, outreach, education, Binodoxys communis, Aphis glycines*

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Males fighting after queen removal in *Chartergellus communis* (Hymenoptera, Vespidae)

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Male's behavior in tropical social wasps from the tribe Epiponini has been poorly studied, because of the difficulty to observe them inside and outside colonies. There are some observations about male's activities during the short period they spend inside colonies. Nevertheless, it is unknown what they do outside colonies and we are completely ignorant about mate behavior and sexual selection processes. For this reason, male's behavior were observed after queen removal in one colony of Chategergellus communis in Brazil. The colony was observed during five months in a farm located in Pedregulho, Sao Paulo. The day after queen elimination, males were observed outside colonies. Males fought aggressively between them, and with workers when they try to get into the nest. Workers behavior against males was similar to the defense behavior used when foreigner individuals tried to get in colonies. Observed males possibly do not belong to the colony, because in a previous study on Chartergellus golfitensis males were observed inside colonies and workers did not behave aggressively with them. Before queen removal no fights were observed between individuals, for this reason we think that males arrived to the colony, in order to try to get in and mate with virgin females. Observations of males' fights are very important because they suggest that males of social wasps compete between them to have access to females, as in other insects. Nevertheless, more studies about male's behavior are necessary to completely understand sexual selection process in social wasps.

Keywords:*social wasps, males behavior*

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Reproduction level of intracytoplasmic parasitic double-strand RNA found in a Camponotus ant is related to host reproductivity

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Liner double-strand RNAs (dsRNA) appear to be ubiquitous in plants and fungi. The dsRNA are also seen intracytoplasmic in insects, being reported in Diptera, Lepidoptera, Orthoptera and Hymenoptera. Sizes of the dsRNA vary among taxa, and it is approximately 6.4 kbp in Hymenoptera. Up to now, the origin and function of the dsRNA is unkown. In this study, we aimed to clarify the relationship between host insect's reproductive states and the multiplication of intracytoplasmic dsRNA. We investigated arborial ants (Camponotus yamaokaii), and the multiplication level of dsRNA in each host caste was examined. Classical phenol chloroform method was applied to abstract nucleic acid. The relative amount of the dsRNA was calculated as the proportion of the amount of focal dsRNA to that of host DNA. The multiplication level of the dsRNA was related to host reproductive status. The dsRNA was significantly large in quantity in the reproductive queens, less large in the virgin queens and major workers, and poor in minor workers and males. Our results suggest that dsRNA adopt the strategy of being abundant in host with good reproductive status, usually reproductive queens, to increase their own fitness. The strategy of dsRNA can minimize extra costs of host ant colonies by avoiding self-reproduction in infertile workers occupying almost all populations in the colonies.

Keywords:*Parasitic double-strand RNA, Social insect, arborial ant*

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The effect of ATM, MRE11, RAD50, NBS1, Ku70 and Ku80 knockdown on DNA repair pathways in cultured silkworm cells

Mie Fujii[1], Hiroaki Mon[2], Masateru Takahashi[3], Hitoshi Mitsunobu[4], Jae Man Lee[5], Takahiro Kusakabe[6]

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Silkworm is known to be highly resistant to ionizing radiation (IR) when compared with mammals. In our previous research, it was showed that the silkworm cell line BmN4 did not undergo apoptosis after γ-ray irradiation at a dose of 100 Gy, which is sufficient to induce apoptosis in mammalian cells. Therefore, we are interested in the responses of silkworm cells against DNA double strand breaks. Double-strand breaks (DSBs) are generally dealt with by homologous recombination repair (HRR) or nonhomologous DNA-end-joining (NHEJ). In silkworm, DNA repair-related genes are highly conserved, but their functions are not well studied. In this presentation, we focused on the silkworm genes related to DSB recognition and repair, such as ATM, Ku70, Ku80, Mre11, Rad50 and NBS1. In mammals, ATM activates DSB response pathways by phosphorylating its substrates, including histone H2AX and 53BP1, which are assembled into chromatin complexes at the sites of DSBs. Human Mre11/Rad50/NBS1 complex plays an important role in the initiation of DNA strand resection required for HRR. The complex recruits ATM/Tel1 checkpoint kinase and CtIP/Ctp1 HRR factor to the DSB sites. Ku70/80 heterodimer is recruited to the DSB sites at the earliest stages of the NHEJ process. In the experiment, we constructed assay systems for HRR or NHEJ in silkworm cells. Using RNAi induced BmN4SID-1 cells, the effect of knockdown of the repair-related genes were analyzed by flowcytometer and the assay systems. Similarly, cell cycle analyses of the knockdown cells following γ-irradiation were performed.

Keywords:*DSBs, HRR, NHEJ, ATM, KU70, KU80, RAD50, MRE11, NBS1*

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Microbial community associated with the red palm weevil, *Rhynchophorus ferrugineus*

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The red palm weevil, Rhynchophorus ferrugineus Olivier, 1790 (Coleoptera: Curculionidae) is one of the most dangerous pest of dates, coconuts and palms. This species, originally distributed in the Southeastern Asia and Malaysia, was recently introduced in the Mediterranean Basin and is now considered a quarantine organism. The xylophagous R. ferrugineus larvae can excavate holes in the trunk of a palm trees, thereby weakening and eventually killing the host plant. The insects with unbalanced diet (i.e. phloem, blood), are usually favored by the presence of symbiotic bacteria in the gut or specialized tissues (i.e. bacteriocytes). Considering the ecology of the red palm weevil, the presence of symbionts that could help the insect development, by the degradation of cellulose or the supply of amino acids or other nutrients, is to be considered. These symbionts may offer a way to impair the reproduction and spread of the arthropods. DGGE investigation on adults, ovaries and testis showed the presence of bacteria belonging to the taxa: Acetobacteriaceae, Burkholderiales and Rhizobiales. TEM examination of ovaries, testis and fat body cells, revealed the presence of free gram-negative bacteria in the cytoplasm of the oocytes. Isolation on traditional media carried out on entire male and female adults and their guts, allowed to isolate bacteria belonging to the taxa: Enterobateriaceae, Acetobacteriaceae and Comamonadaceae. These results are confirming Acetobacteriaceae as important symbionts of arthropods with unbalanced diets. These data are now complemented by FISH microscopy and 16S pyrotag analysis.

Keywords:*Rhynchophorus ferrugineus, microbial community, DGGE, TEM*

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School of Ants: Using global citizen scientist data to map urban biodiversity patterns

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The School of Ants project is a citizen scientist driven study mapping ants in urban areas. This public-academic partnership has two principal goals: scientific discovery and science education. Participation is open to anyone interested in contributing by following a basic collection protocol. Teachers, students, parents, kids, junior-scientists, senior citizens and enthusiasts of all stripes are involved in collecting ants in schoolyards and backyards. These samples form the basis of detailed maps of the wildlife that lives in close association with humans.

Keywords:*Formicidae, citizen science, urban insects, education, ecology, biogeography*

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The Integrated Insect Database System of NIAES (the National Institute for Agro-Environmental Sciences, Tsukuba, Japan)

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Insects are one of the most important components of agricultural ecosystems, and it includes many agricultural pests, benefactors, and bioindicators. Therefore, it is required that development of database system to utilize large amount of insect specimens and data, and to promote accumulation of additional ones. We have been constructing several kinds of database systems concerning insect materials, which are held in NIAES such as specimens, literature, and other data. Recently, to promote utilization of these database, we construct 'The Insect Inventory System' an integrated database system of insect resources and related data kept in the insect museum of NIAES. This system consists of two main pillars of database; one is the taxa information database which contains information accompanying with each categories of insect, spider and plant (mainly host plants of insects) taxa; another is the specimen information database which includes label data (locality, date, collector, etc.), images and DNA information. In the former taxa information database, information of each taxa are linked by interspecific relationship such as hosts, parasitoids, preys and so on. By connecting these databases, we can not only use data and images of specimens, but also various kind of information available for researches. We introduce the characteristic of the system in detail and future strategy to develop it.

Keywords:*database, specimens, taxa information*

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Orthology prediction in a large-scale transcriptome project

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Orthology among amino acid or nucleotide sequences is a prerequisite for inferring phylogenetic relationships of the organisms from which these sequences have been sampled. However, orthology prediction is not a trivial task when working with transcriptome nucleotide data because of their inherent incompleteness. The large amount of data in transcriptome libraries can be an additional challenge for assessing orthology of sequences. Within the 1KITE project (1000 Insect Transcriptome Evolution, http://1kite.org), we assess orthology using a heavily modified version of the HaMStR pipeline (Ebersberger et al., 2009), which maps transcript sequences to pre-established orthologous amino acid sequences. The pipeline uses profile hidden Markov models (pHMMs), which allow for both rapid and specific detection of candidate orthologs, and then verifies orthology of the hits by a reciprocal search against the proteomes of reference species with a completely sequenced proteome. Here, we describe an improved mapping algorithm and its integration in the transcriptome library analysis pipeline of the 1KITE project.

Keywords:*orthology, transcriptome, hidden Markov models, 1KITE*

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Chrysomya megacephala (Fabricius) larvae: A novel bio-resource

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Chrysomya megacephala (Fabricius) is usually noted for a vector of disease, a pollinator in orchards and the forensic adhibition in postmortem interval. We explored the feasibility of using C. megacephala as a novel bio-resource in the present study. Sufficient C. megacephala larvae (CML) were obtained via feeding with a variety of organic wastes. The crude oil content of dehydrated CML powder ranged from 18.1%-34.0% due to variable feeds, while the crude protein content was 45.0%-55.0%. Then, oil was pressed from a CML powder (21.2% crude oil, 54.2% crude protein), and the remained oil content and protein content in the dregs of this CML powder was found to be 4.8% and 67.7%, respectively. The oil (99.8% fat content) property was detected by gas chromatography-mass spectrometry (GC-MS). It was found that CML oil contained 30.62% oleic acid (C18:1), 21.73% linoleic acid (C18:2) and 27.30% palmitic acid (C16:0), which were the overwhelming majority in CML oil. Meanwhile, the dregs were milled into powder in which the amino acids were tested by ion exchange chromatography (IEC). The rate of essential amino acid (EAA) was 39.31%. It was concluded that C. megacephala larvae (maggots) could be used as a novel potential bio-resource for biodiesel , as well as a substitute for fish meal.

Keywords:*bio-resource, Chrysomya megacephala, fish meal, biodiesel*

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Tarsal gustatory sense in coleopteran insects

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It is well known that some insects, such as those from Orthoptera, Diptera, and Lepidoptera, use tarsi as chemosensory organs in feeding and oviposition. However, the role of the tarsus as a chemosensory organ in feeding and/or oviposition is still unclear in insects from Coleoptera, which is the largest order among living organisms. Gustatory discrimination by tarsi in coleopteran insects has yet to be proven directly in ethological experiments, although the electrophysiological and morphological properties of the tarsus have been investigated in a few coleopteran insects such as Chrysomelidae. Firstly, we investigated the existence of tarsal chemosensilla in 89 coleopteran species with a scanning electron microscope. In Chrysomelidae, all genera (species) possessed gustatory sensilla (sensilla chaetica) on their tarsi. In Cerambycidae, gustatory sensilla were observed on the tarsi in 6 of 15 species examined. Secondly, we investigated behavioral responses on tarsal gustation using Galerucella grisescens, which possess tarsal chemosensilla, and Henosepilachna vigintioctomaculata, which possess no tarsal chemosensilla. G. grisescens without maxillary palpi, labial palpi, and antennae could discriminate feeding stimulant, sucrose, in choice tests, but H. vigintioctomaculata could not. That is, G. grisescens could discriminate sucrose by only their tarsi. The insect with only tarsi could also discriminate antifeedant, brucine, and leaf surface wax of host plant. The findings are as follows: 1. In Coleoptera, tarsal gustatory sensilla may develop in only a few groups such as Chrysomeloidea, especially Chrysomelidae. 2. Chrysomelid beetles may use their tarsi as gustatory organs to discriminate their host plants.

Keywords:*tarsus, Coleoptera, Chrysomelidae, gustatory organs, chemosensilla, food discrimination*

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Preparation of chitin and chitosan from the blowfly *Chrysomya megacephala*

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Chitosan, a derivative of deacetylated chitin which is an important constituent of the exoskeleton of many organisms including insects, is widely used in many fields. In this work, chitin was isolated by chemical treatment from the larval cuticle of the blowfly, Chrysomya megacephala, a prevalent necrophagous species easy to be mass reared. The chitin yield (30.04%) was higher than that from traditional commercial sources such as shrimp and crab (20%-25%). The value of nitrogen content (5.78%) was lower than the theoretical value (6.89%) through elemental analysis, implying almost no protein left in this chitin product. FT-IR spectrum showed characteristic peaks of chitin and the absence of peaks at 1540 cm-1 assigned to the stretching vibration of protein, confirming that the chitin product was protein free. The deacetylation degree of chitosan derived from chitin was an average 71%. 13^C CP/MAS NMR spectroscopy revealed slight peak height differences between chitin and chitosan, as well as no peaks directing to organic impurity. The chitin structure appeared as loosely united leaves, while chitosan showed a highly porous structure by SEM analysis. These results suggested that larvae of the blowfly could be a novel alternative source for chitin and chitosan, used for many fields such as agriculture, textiles, cosmetics, medical and food processing.

Keywords:*Chrysomya megacephala, Chitin, Chitosan, Spectroscopy*

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Laccase2 expression involved in the specific cuticular tanning during soldier differentiation of the termite *Reticulitermes speratus*.

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Termite soldiers are morphologically specialized for colony defense. The acquisition of a soldier caste is thought to have occurred just once during the course of termite evolution. Therefore, analyses on mechanisms of soldier differentiation are important for understanding the establishment of termite societies. Soldiers differentiate from workers through a presoldier stage and have well-sclerotized and pigmented cuticles. These characteristics are expected to be caused by soldier-specific mechanisms of cuticular tanning, but their origins have not been resolved. Laccase2 (Lac2) plays important roles in cuticular tanning in insects. Thus, we hypothesized that Lac2 expression was involved in soldier-specific cuticular tanning. We observed inner and outer head cuticles of each caste, and compared the Lac2 expression patterns between soldier differentiation and worker-worker molts in the termite Reticulitermes speratus. First, quantitative analyses of head cuticle colors showed that the hue changed more conspicuously during soldier differentiation than in worker-worker molts. Next, histological observations showed that exocuticules of soldier heads were significantly thicker than those of worker heads. Finally, gene expression analysis revealed that Lac2 was highly expressed just before and after molts to both soldiers and workers, and that the high expression level was kept throughout the presoldier stage. These results suggest that specific cuticular formation occurrs at the exocuticles during soldier differentiation, and that the high level of Lac2 expression during the presoldier stage is related to soldier-specific cuticular tanning. Regulatory mechanisms for Lac2 expression might be important for the acquisition of the soldier-specific cuticles.

Keywords:*cuticular tanning, Laccase2, termite*

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Invasion history of the oriental fruit fly, *Bactrocera dorsalis*, in the Pacific-Asia Region: Two main invasion routes.

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The oriental fruit fly, Bactrocera dorsalis, was initially recorded in Taiwan Island in 1912, and has dispersed to many areas in the Pacific-Asia region over the last century. The area of origin of the species may be confidently placed in South-East China. However, routes of range expansion to new areas and underlying population processes remain partially unclear, despite having been the subject of several studies. To explore the invasion history of this species, a partition of the cox1 gene of mitochondrial DNA was used to investigate genetic diversity, haplotype phylogeny and demographic history of 35 populations, covering China and South-East Asia and including marginal populations from Pakistan and Hawaii. Based on neighbor-joining tree analysis and the distribution of haplotypes, two main invasion routes are inferred: one from South-East China to Central China, another from South-East China to South-East Asia, with both routes probably coinciding in Central China. Populations in Taiwan Island and Hainan Island might have originated in South-East China. The marginal populations in Pakistan and Hawaii might have undergone founding events or genetic bottlenecks. Possible strategies for the control of this species are proposed based on the invasion history and reconstructed expansion routes.

Keywords:*oriental fruit fly, Bactrocera dorsalis, cox1, invasion history, invasion routes*

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Genetic basis of hygienic behavior in *Apis mellifera* (Hymenoptera: Apidae): A candidate gene approach

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The hygienic behavior is described as the ability of the honey bee Apis mellifera (Hymenoptera: Apidae) to detect, uncap and remove diseased brood from the hive. This behavior is performed by middle-aged workers and seems to be one of the main traits involved in the tolerance to several diseases of the hive. The variability of this quantitative trait can be explained by genetic factors but it is also strongly influenced by environmental conditions. The aim of the present study was to explore the genetic basis of hygienic behavior in honey bees by analyzing the gene expression profiles of candidate genes involved in neuronal sensitivity and olfaction. Total RNA and cDNA were obtained from the head of middle-aged hygienic and non-hygienic workers. Gene-expression profiles from each group of bees were obtained using real time quantitative PCR (RT-qPCR) targeting transcripts of AmOa1 (octopamine receptor gene), using the actin gene with a constant expression pattern as a reference. The efficiency for each locus was determined by running a standard dilution series in duplicate. The efficiency of the amplicons for each locus was adequately high and at least 97%. Transcript levels for AmOa1 showed no change between hygienic and non-hygienic bees. Together with the analysis of other candidate genes, already in progress, this result represents a first step toward the understanding of the genetic basis of hygienic behavior in A. mellifera.

Keywords:*honey bee, nest hygiene, behavior genetics, diseases tolerance*

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Progress on Lac research: The processing and utilization

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This paper focused on the status quo of studies on lac processing. Besides lac resin as the main product, it was also summarized on lac red dye as co-product and shellac wax as by-product. During the past decades, techniques were in a stable situation on seed lac, shellac and lac red dye, and it was seldom developed on the producing of bleached shellac. As a traditional field with short industry chain, low conglomeration, simple and crude products, and high pollution load, the processing of lac need urgently to be a clean production with modern separation technology and non-toxic chemicals. It was also mentioned in this review on the application of lac resin for conventional industries such as foods, daily chemicals and coatings. In medicinals and pharmaceuticals, the fine availability made it becoming a new hot research topic. According to properties of the resin and requirments of its application in some specific fields, the modification methods and progressions were proposed both on physical and chemical areas. Chemically, solubility and stability of the resin can be improved by controlled deassociation or polymerization; and the swelling & disintegration velocity can be promoted by decoration with chemical reagents on active groups in the resin such as carboxyl, hydroxyl and aldehyde group. Physically, utilization range of the resin can be widened by the improvement of its film-forming property and dispersion phase. It was pointed out on the prospects of using lac dye in foods and textiles, and using shellac wax in daily chemical industry.

Keywords:*lac, resin, dye, wax, process, utilize*

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Reassembling the transcriptome of the whitefly *Bemisia tabaci* using Trinity

Yu-Jun Wang[1], Xiao-Wei Wang[2], Shu-sheng Liu[3]

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RNA-seq provides an efficient approach to analyze the transcriptome of an organism. However, reconstruction from short raw reads to full-length transcripts still presents a computational challenge, especially for species without a sequenced genome. The Mediterranean species of the whitefly (Bemisia tabaci) species complex (commonly known as Q biotype) is an important invasive pest. Its transcriptome had been sequenced and analyzed using the SOAP de novo assembly method. Recently, Grabherr et al. provided a novel transcriptome assembly method, Trinity, which could reconstruct more full-length transcripts and had higher coverage of reconstructed transcripts per locus. In this study, we reassembled the raw sequencing reads of the whitefly Mediterranean using Trinity method. A total of 181,229 sequences (mean length = 672bp) were obtained and 26,697 sequences (mean length=764bp) were annotated to NCBI non-redundant database with an E-value above 10-5, while by SOAP method, the numbers were 168,900 (mean length = 266bp) and 27,321 (mean length=539bp), respectively. After filtering the sequences that could match to SOAP constructed sequences, we got 28,735 newly discovered sequences. In addition, using Trinity we predicted 9,582 complete coding sequences (CDS) (mean length = 784bp), while the complete CDS were 3,939 (mean length = 631bp) for SOAP. We also compared the sequences assembled by the two methods, and found that more genes related to insecticide targets and metabolisms were annotated using Trinity. Reassembly and annotation of the Mediterranean transcriptome provide additional sequence resources for the functional study of the whitefly.

Keywords:*whitefly, RNA-seq, De novo assembly, transcriptome, next generation sequencing*

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Study on genitalia of female cockroaches of different reproductive types

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Six species of cockroaches belonging to three different reproductive types are examined, meanwhile external structures of female genitalia of oviparous type A, B and ovoviviparous type A are described. The results showed that cockroaches in different reproductive types have distinct differences in the external structures of female genitalia. These differences mainly are: gap between developed paraprocts of oviparous A type cockroaches is very narrow, this structure is conducive to the keel of ootheca was placed in the gap, highly sclerited first valvula is responsible for pushing eggs into ootheca, intersternal fold is opened at both sides; first valvula of oviparous type B with sclerite which is partly covering the second and third valvulae, so can help three pairs of valvulae to flip together, for three pairs of valvulae will turnover down to 90 degrees throughout gestation, intersternal fold is closed at apex only; crosspieces of ovoviviparous type A cockroaches are present and developed, first valvifer arms are highly developed to support brood sac, the whole genitalia is weakly sclerited. It is so inferred that there is a corresponding relationship among the external structures of female genitalia, oothecae and reproductive types, when one of the features known, the other two will be known.

Keywords:*Blattaria, female, reproductive type, ootheca, genitalia*

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Comparative genome analysis of Blattabacterium strains, a bacterial endosysmbiont from omnivorous and xylophagous cockroaches and a termite

Yukihiro Kinjo[1], Seikoh Saitoh[2], Akinori Yamada[3], Masaru Hojo[4], Nathan Lo[5], Hirohumi Watanabe[6], Ian T. Paulsen[7], Liam Elbourne[8], Gaku Tokuda[9]

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Blattabacterium spp. are obligate intracellular bacterial symbionts present in the fat bodies of most cockroaches and a primitive termite. It has long been thought that their central role is conversion of uric acids to usable nitrogenous nutrition for their hosts. Recent publications of genome sequencing of four Blattabacterium strains have demonstrated their abilities to produce amino acids and vitamins from waste urea and ammonia, while genes related to uric acid degradation are lacking. On the other hand, two strains of Blattabacterium from xylophagous Cryptocercus punctulatus (Cpu) and a termite Mastotermes darwiniensis (MADAR) lost some genes for amino acids biosynthesis compared to the other two strains reported from omnivorous cockroaches Blattella germanica (Bge) and Periplaneta americana (BPLAN). Therefore, to examine whether the phylogenetic relationship or a feeding habit of their hosts affected evolution of a gene repertoire of the endosymbiont, we determined the genome sequence of Blattabacterium isolated from Panesthia angustipennis (Paa), which is a xylophagous cockroach but more closely related to the omnivorous cockroach B. germanica than others. The overall synteny of the Paa genome is identical to Bge and its gene repertoire is more similar to Bge, and BPLAN than Cpu or MADAR. Thus, our results suggest that evolution of Blattabacterium genome reflects their phylogenetic relationship rather than feeding habits of the hosts.

Keywords:*Blattabacterium, endosymbiont, genome reduction,*

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Evolution and development of bacteriome symbiosis in the Nysius seed bugs

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Insects are one of the best-studied groups of animals that have evolved intricate endosymbiosis. "Bacteriome" is a highly developed endosymbiotic organ in which an insect harbors its microbial partner in specialized cells called "bacteriocytes". For example, most taxa of the order Hemiptera whose members exclusively feed on plant sap, such as aphids and leafhoppers, possess bacteriomes, and the bacteriome-associated symbionts are essential for development and reproduction of the host insects. Meanwhile, many plant-sucking stinkbugs (Hemiptera; Heteroptera; Pentatomomorpha) are known to harbor bacterial symbionts extracellularly in the crypts of their midgut. The gut symbionts are either beneficial or essential for the host insects. However, a few genera of the family Lygaeidae (seed bugs) do not possess midgut crypts but exceptionally develop bacteriomes that harbor intracellular symbionts (Schneider 1940). Hence, it seems likely that these bug lineages might have evolved the bacteriomes de novo secondarily. The origin of the bacteriomes has been an enigma in the evolutionary and developmental biology. No study has addressed the genetic and molecular mechanisms underlying the bacteriome formation. To tackle this problem, we are studying Nysius seed bugs that possess a pair of red-pigmented bacteriomes in the abdomen. We first characterized a novel symbiont lineage associated with their bacteriomes using molecular and histological techniques. Then, we described the unique organogenesis of the bacteriomes in developing embryos, and also conducted RNAi-mediated suppression of several candidate genes during the embryogenesis. We identified a single HOX gene that is crucial for the development of the bacteriomes. [Supported by BRAIN]

Keywords:*endosymbiosis, bacteriome, Schneideria nysicola, embryogenesis*

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Relative utility of arrhenotokous and Wolbachia-associated thelytokous *Odontosema anastrephae* figitid fruit fly parasitoids for mass rearing and biological control

Andrew Davies[1], Martin Aluja[2], John Sivinski[3]

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Thelytokous parasitoid strains are theoretically advantageous when utilized for biological control, as the absence of males should reduce production costs and potentially increase field efficacy. The maternally inherited intracellular bacterium, Wolbachia pipientis, is capable of inducing reproductive alterations in parasitoids and other arthropods, including parthenogenesis induction, or thelytokous reproduction without males. We utilized indistinguishable sympatric arrhenotokous and thelytokous Odontosema anastrephae Borgmeier (Hymenoptera: Figitidae) fruit fly parasitoid strains to test the relative utility of each reproductive type for mass rearing and biological control. Multi locus sequence typing and antibiotic curing demonstrated that thelytokous O. anastrephae are singly infected with a novel Wolbachia strain likely responsible for parthenogenesis induction. Although uninfected arrhenotokous (W-) O. anastrephae display protandry, average female development time was not different between strains. Estimates of population doubling time were shorter for thelytokous Wolbachia-infected (W+) females when both strains were individually maintained, yet the reverse occurred when strains were reared in groups. This was primarily due to the significantly shorter lifespan of group-maintained W+ females, and despite W- females producing an increased proportion of male offspring when competing for hosts. For logistical reasons, mass-rearing schemes require parasitoids to be reared in groups. Therefore, we believe it is unlikely that Wolbachia-infected thelytokous O. anastrephae would be advantageous relative to uninfected arrhenotokous strains for augmentative release programs.

Keywords:*Wolbachia, Parasitoids, Thelytoky, Biological control, Mass rearing*

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The use appraisal of ant feeding module

Shu-Ping Chen[1], Li-Ling Chao[2], Chung-Chi Lin[3], Yu-Der Wen[4], Su-Hua Lin[5], Wei-Lung Wang[6]

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This study aimed to develop and explore the use appraisal of ant feeding module and to understand the benefits and satisfaction of teachers after used this module. In addition, ants' ecological teaching module integrated into the curriculum and science experiment. A total of 73 junior high, senior high and primary school teachers learn and carry out the experimental course and take a questionnaire. The methods of this study included "The use appraisal and the benefits and satisfaction of teachers used the ant feeding module". The Cronbach's α value of this questionnaire is 0.95 and all of the questions reach significantly difference ($p<0.01$). The data analysis showed that teachers felt very satisfied with the module appearance, it is an attractive design, easy to use, carry, built up, observe, and can also study ant behavior at any time. By the way, it's more important that the experiment not only can enhance a deeper impression on students, but also cause students' motive effectively. In the meanwhile, the students were more like the experimental research of ant. Therefore, it's a quite helpful module in teaching because of its excellent experimental and instructive design.

Keywords:*behavioral and ecological observation module, feeding module*

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First records of new host-parasitoid associations for invasive *Dryocosmus kuriphilus* and successful establishment of parasitoid *Torymus sinensis* in central Italy

Gabriele Rondoni[1], Marco Valerio Rossi Stacconi[2], Carlo Ricci[3], Ferdinando Bin[4], Roberto Romani[5], Gianandrea Salerno[6], Petr Jansta[7], Eric Conti[8]

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The chestnut gall wasp, Dryocosmus kuriphilus Yasumatsu (Hymenoptera: Cynipidae), is native from China and is now considered the key pest of chestnut in the Countries where it has established. In Italy this species was reported for the first time in 2002. Behavioural and morphofunctional studies have been carried out focusing on antennal and ovipositor sensilla. The chestnut gall wasp induces abnormal gall formation, preventing the development of normal shoots, flowers and nuts. The attacks cause an overall weakness of the trees, which seem to become more susceptible to fungal infections. From 2007 to 2012 about 25000 galls were collected from chestnut trees in Central Italy. Overall, 15 species of hymenopteran parasitoids/hyperparasitoids, belonging to Eupelmidae, Eurytomidae, Pteromalidae and Torymidae, emerged from D. kuriphilus galls. The parasitoids Mesopolobus dubius, Torymus longicalcar, Megastigmus nigrovariegatus and Eupelmus vesicularis represent new host records. The most abundant species were Eupelmus urozonus (32% of emerged parasitoids), Mesopolobus amaenus (31%) and Torymus auratus (9%). Although these species may provide remarkable levels of parasitization (overall parasitism rates: 1%-15%) none of them effectively control D. kuriphilus populations. Therefore, in 2011 a biological control program was started in Umbria in collaboration with the Regional Phytosanitary Service and the University of Turin (Alberto Alma), by releasing Torymus sinensis, the most effective parasitoid of the chestnut gall wasp, previously released in Japan, North America and in other areas of Italy. This parasitoid has become established, as confirmed by 2012 samplings.

Keywords:*Chestnut gall wasp, invasive pest, indigenous parasitoid, new association, biological control*

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Development of artificial diets for rearing whitefly larvae (Hemiptera: Aleyrodidae)

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An artificial diet has never been developed for whiteflies larvae. In this study, we tested efficacy of a variety of artificial diets for rearing three whitefly species, Bemisia tabaci B and Q biotypes, and Trialeurodes vaporariorum. Whitefly eggs were collected directly from the leaf surface in water, using a modified version of the Davidson's method (Davidson et al, 2000). The eggs were then isolated using a filter. Hatched eggs were used in the experiments to test the efficacy of a variety of artificial diets on larval development. Whiteflies were fed various concentrations of sucrose solution, which suitable for oviposition onto an artificial membrane, but the B biotype died at the 2nd instar stage. A classical artificial diet for the aphid (Dadd & Mittler, 1966) supported the development of B biotype until the 4th instar. With this diet, the body size at the 4th instar was similar to that when reared on a suitable plant, whereas the developmental period to the 4th instar was delayed by about 5 days, and the 4th instar eye spots never appeared. Those artificial diets were less effective for the development of T. vaporariorum and the Q biotype. In order to determine the requirements for development into adults, we measured the amino acid contents of whiteflies and their host plants using HPLC analysis. We will present the results for alternative artificial diets with modified amino acid contents based on artificial diets used for the aphid.

Keywords:*whitefly, artificial diet*

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Characterization and expression of recombinant alpha-glucosidase III in *Apis cerana indica*

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The α-glucosidase III (HBGase III) was found in hypopharyngeal glands of worker honeybee. This enzyme can hydrolyze sucrose in nectar to be fructose (honey). In order to find the new source of this enzyme, the recombinant technique was used to synthesize interesting enzymes. In A. mellifera (European honeybee), the full length cDNAs of α-glucosidase were cloned and characterization. In contrast, no researches on α-glucosidase were from our Thai native species. Then in this research, it aimed to focus on recombinant HBGase III from A. cerana indica due to economic honeybees. The full length of HBGase III ORF at 1,704 bp was cloned into pPICZαA (an expression vector) and was transformed into Pichia pastoris GS115 (yeast). The expression of recombinant HBGase III was induced by 1% methanol after 144 h. The obtained activity of secreted HBGase III was up to 6 U from the large scale culture (1.2 Liters). In the small scale, secreated HBGase III was purify by Histrap affinity column. Also, pH activity, pH stability, and thermal stability of the enzyme were found to be at 5.0, 4.5-7.5, and <50℃, respectively. Considering substrate specificity, the k0 value for maltose, maltotriose, maltotetraose, isomaltose, p-nitrophenyl α-D glucoside and soluble starch were estimated to be 4.53, 3.87, 2.06, 1.18, 3.33 and 0.59. Also, the Km values for these substrates were 4.5, 22.9, 26.6, 46.5, 4.4 and 11, respectively

Keywords:*α-glucosidase III, Apis cerana indica, substrate specificity, Thai honeybee α-glucosidase*

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Antibacterial activity and chemical compositions of *Apis mellifera* propolis extracts from Nan province, Thailand

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Propolis possesses many bioactivities such as antioxidative, anti-inflammatory, anticancer, and antidiabetic activities. A lot of previous researches reported that those bioactivities of propolis depended mainly on plant sources, bee species, season, harvesting periods, and other external factors. In this research, the propolis of Apis mellifera collected from Nan province was assayed for the antibacterial activity against Staphylococcus aureus (ATCC 20651) and Escherichia coli O157: H7. The crude propolis was extracted by hexane, dichloromethane, and methanol. After that, the activity of these crude extracts were determined by agar well diffusion and microbroth dilution assay. Also, streptomycin was used as a positive control. It was shown that the crude methanol extract of propolis (CME) provided the best antibacterial activity with the minimum inhibition concentration or MIC value of 5 mg/ml and was effective to inhibit both S. aureus and E. coli. Therefore, CME was further purified by quick column chromatography. Three obtained active fractions were effective to inhibit E. coli and S. aureus with MIC values of 6.25 and 31.25 µg/ml, respectively. Later, those pooled active fractions were more purified by adsorption chromatography. These purified fraction 1A was found to be effective to inhibit E. coli with IC50 value of 0.175 µg/ml. In addition, the purified fraction 2 was effective to inhibit S. aureus with IC50 value of 0.004 µg/ml. After being analyzed by Nuclear Magnetic Resonance, it was shown that purified fraction 1A was in a cardanol group and purified fraction 2 will be analyzed in the future.

Keywords:*antibacterial activity, Apis mellifera, agar well diffusion, minimum inhibition concentration, propolis*

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Do the promotional campaigns of firefly watching activities in Taiwan include visitor rules?

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Introduction: As the vigorous development of rural tourism, the firefly watching activities are prevailing during recent decades in Taiwan. These activities provide leisure experiences as well as educational value to visitors by exhibition, watching activity, and observation. But the huge amount of visitation during the occurring season of fireflies has disturbed the mating behaviour of fireflies. Since the pro-visitation education will change the visitor behaviour during firefly watching, the study wants to investigate how the organizers promote the firefly watching activities, in order to unveil why there is improper behaviour of visitors during firefly watching. Methods: The study was conducted a survey using the content analysis. The promotion measures and the contents of the brochure, poster, e-documentation, and press release would be analysis to know if the promotional materials would include visitor rules to educate visitor before they go to watch fireflies. Results: Although the firefly watching activities are prevailing in Taiwan during April to May each year, the main organizers of these activities do not do any effort in pre-education of visitors, hence the disturbance of firefly mating and the habitat emerge in endlessly. Conclusions: Although the great mass fever of firefly watching activities would attract mass visitors during the firefly occurrence seasons, and bring huge economic benefits at the spots of the activities, the visitor behaviour should be improved. The cooperation of activities organizers and entomologist would make conditions much better.

Keywords:*firefly watching activities, visitor rules, promotion*

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Verification of horizontal gene transfer (HGT) of movable element, mariner-like element (MLE), among various insects and spiders inhabiting the South-West Islands of Japan.

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MLEs are derived from a kind of DNA-type transposon, "mariner", and are widely distributed in genomes of various eukaryotic organisms. Past researches have shown that these MLEs have integrated into various genomes by horizontal gene transfer (HGT). In natural population, however, an active HGT event has not been observed yet. In this study, we compared MLEs isolated from various species of Araneae (spiders), Hymenoptera and Lepidoptera in the South-West Islands of Japan. Varieties of species inhabit the narrow islands and such high density of species makes it suitable site for HGT analysis of MLEs. MLEs were isolated by the PCR amplification using five specified inverted terminal repeats (ITRs) for various known MLE types as primers. As a result, a mellifera-type MLE was found to be the major MLE type from those insects and spiders inhabiting the South-West Islands of Japan. The mellifera-type MLEs isolated from various spiders and insects were divided into two clusters (A and B) on a phylogenetic tree by Neighbor-Joining (NJ) method. The MLEs classified in cluster A showed very high homology (>92%) among them. Therefore, it is suggested that HGT of the mellifera-type MLE might occurred among those closely-inhabiting species. In addition, some clones containing a full open reading frame (ORF) encoding transposase were isolated from wasps and spiders. These ORFs showed high homology (91-96%) to that encoding functional trasnposase from Forficula auricularia. In species inhabiting the South-West Islands, the spreading and invading of the mellifera-type MLE seems to be a recent or present event.

Keywords:*transposon, mariner-like element, horizontal gene transfer, phylogenetic analysis, wasp, spider*

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Feeding humans with edible insects : actual state and perspectives in Belgium and Europe

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In future decades, world population will grow up to 9 billion of people and we will be confronted to a lack of nutritive resources. We will not continue to produce proteins with our conventional livestock as beef, poultry or pig. It will therefore look to other sources and edible insects are one of these solutions. Indeed, more than 1400 species of edible insects were actually consumed by 3500 ethnic groups in the world. In undernourished populations, entomophagy is essential to relieve deficiencies in proteins, fatty acids and some vitamins. In Europe, we have acquired sedentary habits and we have lost our ancestral harvesting and hunting traditions. It is the reason of disinterest for edible insects and entomophagy was considered as a 'barbarian' food habit. Facing food challenges of tomorrow, it is important to sensitize industrialized populations and to reintroduce edible insects in our plates and habits. The first step is to overcome neophobia of food products. Our studies focused on different insect preparations and on perception of entomophagy by different age classes. Globally, our first results indicated that entomophagy was accepted by belgian consumers but the more difficult for them is to taste the first time. These results confirmed neophobia for this type of food products and thus the importance of positive informations and education for acceptance of entomophagy.

Keywords:*entomophagy, eadible insect, neophoby, feeding behaviour, human*

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Using species distribution model to assess potential invasion risk of phytophagous pests - a case study of *Leptocybe invasa* in Taiwan

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The aim of this study is using species distribution model (SDM) to model invasive phytophagous pests for early warnings and preventing possible damage in advance. An internationally important pest: Leptocybe invasa (Eulophidae) was selected for this study. The world-wide occurrences of this species were used for projecting potential distributions in Taiwan under current and two climate change scenarios (a2a and b2b at year 2050 and 2080, Worldclim database, CCCMA model). The SDM results indicated that L. invasa had a high risk of invasion under current and both climate change scenarios. The most important factors for its distribution were the lowest temperature of the coldest month (31.3%), the rainfall of the coldest season (14.8%) and isotherm (13.6%), and the western plain of Taiwan was its potential suitable habitat. The projection was done in 2009, and we recorded L. invasa in the projected area soon after year 2010, which supported this study result. This study indicated that SDM is a useful tool for assessing potential invasion risk, and provides valuable information on pest quarantine.

Keywords:*invasive species, distribution model, climate change*

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Optimization of fermentation conditions of mulberry wine by response surface methodology

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In this study, the mulberry (Morus alba L.) fruit was utilized as raw material to brew fruit wine. The effects of fermentation parameters (inoculation amount, fermentation temperature, fermentation time and pH value) on the alcoholicity of mulberry wine were investigated systematically using response surface methodology, and analyzed by the design expert 7.1 software. Based on single factor and three factor influence level tests by following the Plackett-Burman design, the optimum extraction yield was analyzed by response surface methodology (RSM). The results of RSM showed that the optimal condition for mulberry fermentation was defined as pH 3.2, inoculation amount 0.53%, fermentation temperature 31.4ºC, and fermentation time 6 d. Under this optimal condition, the alcoholicity was 12.46% (V/V).

Keywords:*mulberry wine, fermentation conditions, response surface analysis, optimization*

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Improved 1-Deoxynojirimycin (DNJ) production in mulberry leave fermented by microorganism

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DNJ, an inhibitior of α-glucosidase, is used to suppress the elevation of postprandial hyperglycemia. In this study, we focus on screening an appropriate microorganism for performing fermentation to improve DNJ content in mulberry leaf. Results showed that Ganoderma lucidum was selected from 8 strains and shown to be the most effective in improvement of DNJ production from mulberry leave through fermentation. Based on single factor and three factor influence level tests by following the Plackett-Burman design, the optimum extraction yield was analyzed by response surface methodology (RSM). The extracted DNJ was determined by reverse-phase high performance liquid chromatograph equipped with fluorescence detector (HPLC-FD). The results of RSM showed that the optimal condition for mulberry fermentation was defined as pH 6.97, potassium nitrate content 0.81% and strain volume 2.00 ml. The extraction efficiency reached to 0.548 % in maximum which is 2.74 fold of those in mulberry leaf.

Keywords:*mulberry, microorganisms, fermentation, DNJ, production*

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Tandem repeat markers as novel diagnostic tools for high resolution fingerprinting of *Wolbachia*

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Strains of the common endosymbiotic bacterium Wolbachia pipientis are extremely diverse both genotypically and in terms of their induced phenotypes in invertebrate hosts. Despite extensive molecular characterisation of Wolbachia diversity, little is known about the actual genomic diversity within or between closely related strains that group tightly on the basis of existing gene marker systems, including Multiple Locus Sequence Typing (MLST). The genome of the wMel strain of Drosophila melanogaster contains inter- and intragenic tandem repeats that may evolve through expansion or contraction. We identified hypervariable regions in wMel, including intergenic Variable Number Tandem Repeats (VNTRs), and genes encoding ankyrin (ANK) repeat domains. We amplified these markers from 14 related Wolbachia strains belonging to supergroup A and were successful in differentiating size polymorphic alleles. Because of their tandemly repeated structure and length polymorphism, the markers can be used in a PCR-diagnostic multi locus typing approach, analogous to the Multiple Locus VNTR Analysis (MLVA) established for many other bacteria and organisms. The isolated markers are highly specific for supergroup A and not informative for other supergroups. However, in silico analysis of completed genomes from other supergroups revealed the presence of tandem repeats that are variable and could therefore be useful for typing target strains. These novel inexpensive high-throughput fingerprinting tools will be useful for studies of population genetics, horizontal transmission and experimental evolution of Wolbachia.

Keywords:*Wolbachia, insect symbiosis, DNA fingerprinting*

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Study on the key technique of mass production of *Encarsia formosa* in greenhouse

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Encarsia formosa is well known as an important parasitoid of greenhouse whithfly,and has been used successfully in many countries throughtout the world. In China, the research of production and application of Encarsia formosa has been done in control of greenhouse whithfly since 1980s. The key techniques of natural enemies mass rearing, are important for the capacity and quality of the products,which worked in building an mass production line and procedure.The study on the production and applicaition of Encarsia formosa have been studied in our institute for ten years. Some research works of Encarsia formosa production , such as host-plant selection, the whiteflies adult cleaning-up and parasitized whitefly pupae collection, were studied on the production line in greenhouse. We have primarily found the key techniques and process of mass rearing of Encarsia formosa in general greenhouse,which are significant to promote the capacity and quality of products.Also we have build a year-round quality control procedure and standard of the Encarsia formosa,to ensure the the natural enemy could be good used in the greenhouse field.

Keywords:*Encarsia formosa, Mass production, Key technique*

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Improving production of toyocamycin in Streptomyces by metabolic engineering

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The biopesticide has previously shown its high potential in controlling agricultural harmful pests, which was usually produced as secondary metabolites by microorganism. In our research group, a strain Streptomyces diastatochromogenes1628 capable of producing toyocamycin as a member of nucleoside antibiotics family was isolated. The inhibitory activity of toyocamycin against many pests has been proved after repeated field experiments. All the time the research progress on biosynthesis of toyocamycin was slow because of unclear molecular mechanism and metabolic pathways of toyocamycin. Therefore, it limited scale-up industrial application of toyocamycin. In order to improve the production of toyocamycin of strain 1628, we optimized and constructed the genetic transformation methods and expression system for strain 1628. The vhb gene encoding bacterial hemoglobin was successful heterologous expression in 1628, the recombinant showed the 2-fold increase in the production of toyocamycin with respect to control in primary fermentation experiment. Moreover, we constructed the gene library of strian 1628, and cloned the four key genes in gene cluster required for the biosynthesis of toyocamycin. In order to elucidate the biosynthesis and regulation mechanism of toyocamycin in microorganism at the molecular level, the function of genes or gene cluster via the genomics and transcriptomics methods will be our future work. All these will pave the way as well as supply abundant materials for future construction of engineering strain with improved toyocamycin production.

Keywords:*Toyocamycin,biosynthesis, metabolic engineering*

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Overexpressed esterases in a fenvalerate resistant strain of the cotton bollworm, *Helicoverpa armigera*

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Enhanced detoxification is the major mechanism responsible for pyrethroid resistance in Chinese populations of Helicoverpa armigera. Previous work has shown that enhanced oxidation contributes to resistance in the fenvalerate-selected Chinese strain, YGF. The current study provides evidence that enhanced hydrolysis by esterase isozymes also contributes to the resistance in this strain. The average esterase activity of third instar YGF larvae was 1.9-fold compared with that of a susceptible SCD strain. Much of this difference was attributed to isozymes at two zones which hydrolysed the model carboxylester substrate 1-naphthyl acetate and also a 1-naphthyl analogue of fenvalerate. A preparation enriched for enzymes migrating to one of these zones from YGF was shown to hydrolyse fenvalerate with a specific activity of about 2.9 nmol/min/mg. This material was also matched by mass spectrometry with four putative carboxylesterase genes, all of which clustered within a phylogenetic clade of secreted midgut esterases. Quantitative PCR on these four genes showed several-fold greater expression in tissues of YGF compared to SCD but no differences was found in the number of copies of the genes between the strains.

Keywords:*Carboxylesterase, Pyrethroids, Metabolic resistance*

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Spinosad resistance in the olive fly, *Bactrocera oleae*

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The olive fruit fly Bactrocera oleae is the most important and destructive pest of cultivated olive trees, in almost every country where olive trees grow. For the last 40 years, the management of the pest has been based on the use of organophosphate insecticides. More recently, pyrethroids and the naturalyte spinosad have been added in the arsenal against the olive fly. However, the extensive use of any insecticide inevitably leads to the development of resistance. Low level spinosad resistance to the olive fly has been demonstrated in flies caught in several California counties where the drug is the only insecticide used for the control of the fly. In Drosophila, the α6 subunit of the nicotine acetylcholine receptor (nAChR) has been implicated in spinosad resistance. On the contrary, the α6 subunit of the nAChR does not seem to be spinosad's target in Musca domestica. In order to investigate the mechanism of spinosad resistance in the olive fly, we developed a ~30-fold spinosad resistant strain in the laboratory under continuous selection. By virtue of homology we isolated B. oleae α6 nAChR both from the laboratory sensitive and resistant strains in an effort to identify possible sequence variants in this locus that could be responsible for the observed resistance. At the same time, whole transcriptome analysis was performed in heads of the sensitive and resistant strains in order to study the differential expression of the two strains and identify the loci involved in spinosad resistance.

Keywords:*spinosad, insecticide, resistance, olive fly, Tephritidae, nicotine acetylcholine receptor, transcri*

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Exploring miRNA-mRNA interactions of the honeybee embryonic development

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The current knowledge about honeybee early development is mainly composed by morphological descriptions used to properly stage embryos. Only few studies investigated gene expression of some Drosophila melanogaster orthologs known as playing a role in insect development. Our group aims to describe molecular determinants governing major process of embryonic period. In particular, we herein investigated the expression of coding and non-coding transcripts in developing female embryos in order to verify their abundance and regulatory associations. Thus, we built and deep-sequenced mRNAs and small RNAs libraries for five embryonic developmental phases: clivage, blastula, gastrulation, germ-band extension and retraction, and larval body formation. Pearson correlation (r) was calculated to identify coding genes and miRNAs with antagonistic transcriptional profiles ($r < -0.8$). More than seventy thousand pairs of miRNA-mRNA showed negative correlation throughout five developmental stages analyzed. Then, we searched for miRNAs binding-sites in 3' UTR of those mRNAs using RNAhybrid software. The analysis resulted in a highly-connected regulatory network containing 318 miRNA-target interactions. The miRNAs miR-34, miR-932, miR-10, miR-92b emerged as potential regulators of neural development, since they seems to regulate expression of RhoGAP93B, puc, Rfx, esg, Tor, and ort. Our results also pointed Ikb1, which is known as a positive regulator of apoptotic process, as target of miR-263a. In Drosophila melanogaster, miR-263a donwregulates pro-apoptotic genes. Apoptosis is an important process for neurogenesis since it reduces the number of neuroblasts present in embryonic ventral nerve cord. These findings provide the first transcriptomic insights into mechanisms underlying honeybee embryogenesis.

Keywords:*Embryogenesis, Honeybee, Transcriptome*

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Who rules the colony? A case study in neotropical social wasps (Hymenoptera, Vespidae: Epiponini).

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Wasps of Epiponini tribe are polygynic and many species do not show morphological differences between 'queens' (reproductive females) and 'workers' (non-reproductive females) because castes may be determined by competitive interactions among the individuals of the colony. These conflicts may be reflected in behavioral interactions, such as those observed during egg-laying behavior. Oviposition was recorded in four colonies of Synoeca surinama (Linnaeus) and analyzed statistically. Colonies in the worker production phase showed higher rates of emergence of young and spent more time on Egg Licking and Vigilance behavior, suggesting that more conflicts were happening. The mean of time dedicated to egg-laying was of 186.97+-5.64s for all colonies and no significant difference was noted among them. In the establishment phase colonies, some workers were observed usurping queens during the Egg Licking behavior, which may be a behavior related to the reproductive control by the older workers. Our findings suggest that a subset of older workers are the real pace marker of the colony, stimulating worker behavior and policing reproduction.

Keywords:*Worker Control, Queens, Social Wasps, Competition.*

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Flower power? Effects of apple orchard groundcover management on arboreal spiders (Araneae)

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Companion plants provide alternative prey, shelter and overwintering habitat for arthropod natural enemies and thus might increase their abundance and efficacy in pest control in agricultural fields. We report results of a six-year study on the effects of annual and perennial flowering herbs sown in alleys of an apple orchard (FLOWER) on the arboreal spider communities and their prey. Plots with weed-free bare ground (BAREgr) and with regularly mowed grass (GRASS) served as controls. Altogether 4309 individuals comprising 45 species were collected in the canopy of apple trees. The abundance and diversity of spiders increased in the presence of flowering ground cover plants. The dominant spider species was Carrhotus xanthogramma (Salticidae) representing 55% of the total catch. Its abundance was 3.5 times greater in FLOWER than in BAREgr with GRASS between. Most of the other spider species showed similar pattern. When the correlation between different spider groups and their potential prey was examined, no connection was found between the abundance of apple pests [green apple aphids (Aphis spp.), apple feeding canopy insects (heteropterans, coleopterans, leafhoppers), leafminer moths and fruit injury caused by Cydia pomonella (Lepidoptera: Tortricidae) and Adoxophyes orana (Lepidoptera: Tortricidae)] and the abundance of C. xanthogramma, non-C. xanthogramma spiders and total spider community. However, the spider abundances were positively correlated with the abundance of hymenopteran parasitoids, dipterans and leafhoppers, planthoppers and froghoppers (Auchenorrhyncha). We concluded that enhancement of spiders through habitat management did not lead to increased pest suppression.

Keywords:*Apple orchard ground cover, Flowering herbs, Habitat manipulation, Spiders, Araneae*

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First record of deer ked (*Lipoptena cervi* (Diptera, Hippoboscidae))-infestation on the red deer in Hansag Region (Hungary)

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Prior to our analyses we did not find any data on the deer ked infestation in the red deer in the Hansag Region(the North-West of Hungary).Between 17th November 2010 and 2nd November 2011 we examined 31 red deer all of which were shot on hunting.The aim of our observations was to declare the incidences and rates of deer ked.The investigations were carried out at the site of shooting or after the transportation of the deer, in a cold storage.They were always examined on the same region and territory of the right part of the neck (on the calves: 620,on the hinds:890 and on the stags 1440 cm²).The coats of the deer were thoroughly combed and the ked found on the animals were afterwards fixed and stored in 10% alcohol. As a result of the identification(by Soos 1995, and Kaunisto et al.,2009,with help of the Conrad USB microscopic camera(1,3 Mpix)of 1994 deer ked, the incidence of genders was found on 1113 for female and 881 for male exemplars.The quantitative parasitological data(using the QP 3.0method(Rozsa,Reiczigel et al 2000,2005))covered:the host sample size:31; the number of infected hosts in the sample:28,prevalence: 90.3%,min. intensity:1, and max. intensity:238. The confidence limits of prevalence (P=95%):0.74-0.97,mean intensity:71.21,the confidence limits of mean intensity (P=95%):48.61-102.36, median intensity: 46.5, the confidence limits of median intensity (P=95%):20-78. The index of discrepancy was: 0.551.

Keywords:*Lipoptena cervi-infestation, QP 3.0, Hansag, Hungary*

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Others

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Termination of expression of BmEP80 protein is responsible for the lethal egg of "Ming" mutant of the silkworm, *Bombyx mori*

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The l-em mutant is a lethal egg mutant from the silkworm (Bombyx mori) variety Ming. Its eggs lose water and become concaved in an hour. To study its molecular mechanism, we used SSR molecular marker technology to map the l-em gene in a region of 0.36 Mb containing 24 genes in the 10th chromosome. Fluorescence quantitative analysis of these genes showed that the expression of gene BGIBMGA006755 was reduced sharply and expression of gene BGIBMGA006756 was inhibited completely in ovarian of the mutant pupa, while the expression levels of other genes did not change between the mutant and wild type. Gene BGIBMGA006756 encodes BmEP80 protein which is a vitelline membrane protein and plays an important role in maintain the structural integrity of eggshell. Inverse PCR showed that the sequence from 3'UTR of gene BGIBMGA006755 to the forepart of gene BGIBMGA006756 was cut off and instead by new fragments which resulted in destroy of the 3'UTR of gene BGIBMGA006755, the promoter and part ORF of gene BGIBMGA006756. 2-D analysis showed that BmEP80 protein expressed from the ninth day of pupa to eclosion in wt, but no expression was detected in the mutant. The results of SiRNA interference against BGIBMGA006755 and BGIBMGA006756, respectively in pupa stage showed that expression of gene BGIBMGA006756 was reduced and the eggs became depression to some degree after SiRNA interference. Thus we speculated that the termination of expression of BmEP80 protein is responsible for the l-em mutation. *Acknowledgments The National Natural Science Foundation of China （No. 31072083）

Keywords:*Bombyx mori, l-em, mutant, SSR, SiRNA interference, BmEP80 protein*

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Insect imaging at ANKA: methods, recent developments and perspective

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Non-destructive imaging techniques used to investigate the 3D morphology of biological samples include confocal laser scanning microscopy (CLSM), magnetic resonance imaging (MRI) and X-ray computed microtomography (µCT). While CLSM is restricted to transparent, fluorescent objects, MRI and µCT can be used to visualize non-translucent organisms. In µCT, X-rays emitted by a synchrotron light source provide a wide energy range, high photon flux as well as small source size and beam divergence, thus allowing analyzing even millimetre-sized samples like small insects in detail. At the ANKA synchrotron light source several studies on insect morphology were performed recently. Experiments included the investigation of the functional morphology of beetle hip joints (van de Kamp et al. 2011) and the examination of amber inclusions of fossil weevils (Riedel et al 2012). At present we are developing a fully-automated setup for high-speed tomo-graphy, including sample exchanger, on-line assessment and data-driven process control. This new system will allow a high sample throughput and facilitate even large comparative studies on insect morphology. The setup will be integrated into the IMAGE beamline that is currently being constructed. Here, we will give an overview about recent experiments and visualization techniques and provide an outlook for future developments at ANKA.

Keywords:*ANKA, synchrotron X-ray microtomography, high-speed imaging*

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Force balance in butterfly take-off

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Up to now, take-off maneuvers remain an elusive phase of flight relatively poorly explored compared to other maneuvers, such as the forward flight or stationary flight. It is a key stage, particularly interesting since it requires a fast production of a significant amount of vertical force to counterbalance body weight and lift it up vertically. Mechanisms involved in this force production during this phase have rarely been explored. Focusing on the first downstroke, we have addressed this problem in butterflies taking-off from the ground. In order to determine if the sole aerodynamic force produced by the wings could explain the observed motion of the insect body, we have compared a rough estimate of the force produced by the wings to the acceleration of the center of mass of the insect calculated from the video tracking of the body motion. Numerical simulations were then performed to decipher the role of ground effect in take-off flight. The vertical force acting on the insect often presents a spike earlier in take-off flight, while the wing force is still at a zero value, in addition to the peak happening around the one occurring in the aerodynamic wing force. Numerical simulations highlight that ground effect cannot be responsible for it, suggesting instead a role of legs propulsion in the initiation of the maneuver.

Keywords:*Take-off, Butterlfy, Forces*

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Bumblebee venom serine protease and serine protease inhibitor affect blood coagulation and fibrinolysis

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Bee venom contains serine proteases and serine protease inhibitors. In this study, we identified a bumblebee (Bombus ignitus) venom Kunitz-type serine protease inhibitor (Bi-KTI) that acts as a plasmin inhibitor. Bi-KTI showed no detectable inhibitory effect on factor Xa, thrombin, or tissue plasminogen activator. In contrast, Bi-KTI strongly inhibited plasmin, indicating that it acts as an antifibrinolytic agent. The fibrin(ogen)olytic activities of B. ignitus venom serine protease (Bi-VSP) and plasmin in the presence of Bi-KTI indicate that Bi-KTI targets plasmin more specifically than Bi-VSP. These findings demonstrate a novel mechanism by which bumblebee venom affects the hemostatic system through the antifibrinolytic activity of Bi-KTI and through Bi-VSP-mediated fibrin(ogen)olytic activities, raising interest in Bi-KTI and Bi-VSP as potential clinical agents.

Keywords:*Bumblebee, Antifibrinolytic agent, Plasmin inhibitor, Serine protease inhibitor, Venom*

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Bumbleee (*Bombus terrestris*) venom serine protease inhibitor acts as a plasmin inhibitor

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Bee venom is a rich source of pharmacologically active substances. In this study, we characterized a B. terrestris venom Kunitz-type serine protease inhibitor (Bt-KTI). Bt-KTI consists of two exons encoding 82-amino acids (aa), including a predicted 24-aa signal peptide and a 58-aa mature peptide. Recombinant Bt-KTI was expressed as a 6.5-kDa peptide in baculovirus-infected insect cells. Bt-KTI showed no detectable inhibitory effect on factor Xa, thrombin, or tissue plasminogen activator. In contrast, Bt-KTI strongly inhibited plasmin, indicating that it acts as a plasmin inhibitor. The electrophoretic mobility shift assay showed that Bt-KTI binds to plasmin, indicating the formation of a plasmin-Bt-KTI complex. These results demonstrate that Bt-KTI acts as an antifibrinolytic agent, suggesting a role for Bt-KTI as an anti-bleeding agent.

Keywords:*Bombus terrestris, Antifibrinolytic agent, Plasmin inhibitor, Serine protease inhibitor, Venom*

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Molecular characterization of a spider (*Araneus ventricosus*) major ampullate silk protein

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Spider silks hold great potential as biomaterials with extraordinary properties. Here we report cloning and characterization of the major ampullate silk protein gene from the spider Araneus ventricosus. A cDNA coding for the partial major ampullate silk protein (AvMaSp) was cloned from A. ventricosus. Analysis of the cDNA sequence shows that AvMaSp consists of 240 amino acids of a repetitive region and 99 amino acids of a C-terminal non-repetitive domain. The peptide motifs found in spider major ampullate silk proteins, (A)n , (GA)n, and (GGX)n, were conserved in the repetitive region of AvMaSp. Phylogenetic analysis further confirmed that AvMaSp belongs to the spider major ampullate spidroin proteins. The AvMaSp-R cDNA, which contains sequences encoding for 240 amino acids of a repetitive domain, was expressed as a 22 kDa polypeptide of soluble form in baculovirus-infected insect cells. Recombinant AvMaSp-R was degraded abruptly by trypsin. However, AvMaSp-R was stable at 100 °C for at least 30 min. Additionally, the AvMaSp-R was stable at various pH values from 2 to 12 for at least 1 h. Taken together, our findings provide the molecular structure and biochemical property for A. ventricosus major ampullate silk protein as a biomaterial.

Keywords:*Araneus ventricosus, baculovirus expression vector, major ampullate silk protein, spider*

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Which between acetylcholinesterase 1 and acetylcholinesterase 2 plays the major role in synaptic transmission in the Class Insecta?

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Most insects possess two different acetylcholinesterases (AChE1 and AChE2) but it remains unknown which AChE plays the major role in synaptic transmission. To investigate the evolutionary distribution of AChE1 and AChE2, the AChE with the main catalytic function in several insect species belonging to 18 representative orders was determined by native-PAGE in conjunction with Western blotting using AChE1- and AChE2-specific antibodies. Among the 98 insect species examined, AChE1 was expressed as the main enzyme in 65 species across diverse taxa. In the remaining species, however, AChE2 was expressed as the major enzyme. These findings are contrary to the common expectation that AChE1 is major enzyme in most insects, with the exception of Cyclorrhapha, and further demonstrate that the specialization of AChE2 as the main enzyme or the replacement of AChE1 function with AChE2 function were rather common events, having multiple independent origins during insect evolution. It was hypothesized that the generation of multiple AChE2 isoforms via alternative splicing has allowed the loss of ace1during the functional replacement of AChE1 with AChE2 in Cyclorrhapha. However, the presence of AChE2 as the main AChE in higher social Hymenoptera provides an example of the functional replacement of AChE1 with AChE2 without the loss of ace1. The current findings should provide valuable insights into which AChE has evolved to perform synaptic functions and to become the main insecticide target in different species.

Keywords:*acetylcholinesterase, evolution, insect, functional replacement*

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Availability of sugar solutions for colony development of the European bumblebee, Bombus terrestris

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Bumblebees are widely used to pollinate various crops, especially tomato, in greenhouses and fields. Here, we investigated whether different sugar solutions have any effects on the oviposition and colony development of Bombus terrestris queens. The types of sugar solution used were a white sugar solution, brown sugar solution, dark brown sugar solution, and prepared sugar solution; all solution contained with a 40% sugar concentration and 0.3% sorbic acid. Among these sugar solutions, B. terrestris queens reared on the white sugar solution exhibited the best results; the rates of oviposition, colony foundation and progeny-queen production were75.0± 11.0, 33.0 ± 9.6 and 21.7 ± 7.3, respectively, which corresponded to 1.1-33.0-fold increases over the results of other sugar solutions. Queens reared on the prepared sugar solution did not found any colonies. The death rate within one month was lowest for the queens reared on the white sugar solution at 26.1 ± 10.9%, which was 1.2?2.0-fold lower than the rate for the other sugar solutions. Furthermore, the numbers of progeny produced by queens reared on the white sugar solution, 131.4 ± 38.8 workers and 51.0 ± 40.6 queens, were also higher, corresponding to 1.1-1.2-fol d increases compared to queens raised on the other sugar solutions. Therefore, the white sugar solution was the favorable sugar solution for the egg-laying and colony developmental characteristics of B. terrestris queens.

Keywords:*Bumblebee, Bombus terrestris, Sugar Solutions, Colony Development*

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A study on the development of functional cosmetics using silk-gland powder of silkworm

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In this study, silk-gland powder of silkworm were investigated to see the possibility for functional cosmetics materials. To test possibility as a functional cosmetic materials, total content rate of amino acids, DPPH free radical scavenging assay, MTT assay, and clinical trial were done. According to the result of the analysis of the amino acids of silk-gland powder, serin (26.77%) content was the highest and asparatic acid, glycine, and glutamic acid were followed. DPPH free radical scavenging activity of silk-gland powder was lower than vitamin C by 82.3% and 97%, respectively, which is relatively good. The moisture effect were increased in twincake with silk-gland powder and baby powder with silk-gland powder compared to control cosmetics by 257.2% and 50%, respectively. Elasticity effect were increased in twincake with silk gland powder compared to control cosmetics by 181.2%. Also, baby powder with silk gland powder was classified as a practically non-irritating material based on the score 0.05 of primary irritation index. Thus, these results suggest that silk-gland powder of silkworm may have beneficial properties as a material for cosmeceuticals.

Keywords:*Bombyx mori, silk-gland powder, moisture, antioxidative activity, cosmetics*

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Effects of mulberry leaves as the supplements to weaning pig diet

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This study was conducted to develop natural alternative antibiotics for pig production from mulberry leaves. A total 140 weaning pigs (6.66±0.4kg average body weight) were assigned to 4 treatments, 4 replicates and 9 pigs per pen in a randomized complete block design. Treatments were 1) NC (basal diet), 2) AB (basal diet+antibiotics), 3) LMB (basal diet+1% melberry leaves), and 4) HMB (basal diet+2% melberry leaves). During the whole experimental period, average daily gain (ADG) of treatment AB (602g) was higher than other treatments. However, LMB treatments was higher in feed efficiency (FE) by AB. Threfore, mulberry leaves was likely able to improve the growth performance, and reduce feed cost although they do not have similar effects like antibiotics in weaning pigs. Pigs fed with mulberry leaves (LMB and HMB) had lower total solid and volatile solid than AB. In the case of E.coli and Salmonella, the LMB inhibited their number in feces. These results suggest that the dietary mulberry leaves are likely able to improve the growth performance, occurrence of diarrhea and immune response although they do not have similar effects like antibiotics in weaning pigs.

Keywords:*Bombyx mori, silk-gland powder, moisture, antioxidative activity, cosmetics*

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Factors of color variations of elytra of *Harmonia axyridis*(Coleoptera: Coccinellidae)

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Harmonia axyridis has been called the multicolored Asian ladybird beetle and showed a high level of phenotypic polymorphism in elytra color pattern within species. Up to now, there is no evidence to indicate whether the variation is genetic and/or environmental factors. Elytra color patterns of H. axyridis were classified into 5 main patterns (succinea 1, succinea 2, conspicua, spectabilis, and axyridis) according to ground color, color of marking, number of spots, and fusions between the spots. As regional results of occurrence proportions in 5 main patterns annually, succinea patterns were more collected than any other type at the rate of 90% and over. There were differences between two differential environmental condition areas on elytra color expression patterns. Specifically, it was investigated that occurrence rate of melanic patterns(conspicua, spectabilis and axyridis) relatively increased in overwintering populations collected in highly mean temperature and longer cumulative day length area. For confirming differences in their genetic constitutions of each color pattern, this study used AFLP analysis so that many segregating markers were generated among the each color pattern. Based on the sequences of colorpattern-specific AFLP markers,primers were designed for PCR amplification of genomic DNA and the AFLP markers converted into SCAR markers.

Keywords:*Harmonia axyridis, Polymorphism, AFLP*

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Honeybee visiting and Nectar Characteristics on Two Species of Genus *Tilia* as a honey plant

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The principal objective of this study was to evaluate the efficacy of Tilia amurensis and Tilia manshurica as honey plants. Flowering period, number of flowers, honeybee wisiting, and nectar secretion were measured. The flowering period of T. amurensis was 9 days, from June 9th to June 17th, and the flowering period of T.manshurica was 11 days. from June 17th to June 27th. The estimated total number of flowers per individual tree was shown to differ by more than 6-fold between the two species T.manshurica was estimated to produce 386,000 flowers and T.amurensis was estimated to produce 63,600 flowers. Both species evidenced two peaks per day in honeybee visiting, and the total honeybee visiting scores of the two species did not differ greatly. The volume of nectar secretion, however, did differ noticeably between the two species T. amurensis secreted 3.58±0.97㎕ and T.manshurica secreted 18.4±2.01㎕.

Keywords:*Honeybee visiting, Tilia amurensis, Tilia manshurica, nectar*

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Behavioral response of western flower thrips to visual and olfactory cues with Chrysanthemum

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Behavioral responses of Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) to visual and olfactory cues were assessed in a 4-way choice chamber and a Y-tube olfactometer. Frankliniella occidentalis preferred yellow flower to other color patterns (White and pink) in greenhouse. In pair wise choice test, Y-tube olfactometer test, the thrips preferred white flower to other color patterns (yellow, red, violet). But In multiple choice test, 4-way choice olfactometer test, the thrips preferred yellow color to other color patterns. Higher numbers of thirps responsed to visual and olfactory cues in chrysanthemum but visual cues higher then olfactory cues. These results suggest that color pattern is important cue for F. occidentalis.

Keywords:*Frankliniella occidentalis, Olfactometer, Chrysanthemum*

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Qualitative assessment of outbreak risk and consequences of introduction of *Bactrocera invadens* through export of fresh fruits of *Citrus sinesis* from Nigeria

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University of Ibadan, Nigeria

The biology of Bactrocera invadens is reviewed in relation to the phytosanitary risks associated with export of fresh fruits of Citrus sinesis from Nigeria to the United States of America where the pest is not known to occur nor widely established; although other closely related fruit flies especially the Mediterranean fruit fly, Ceratitis capitata already exists. This pest, B.invadens is polyphagous and can survive in all tropical and sub-tropical conditions. It poses a potential risk to established orchards in the states of Florida, Texas and Alabama as well other states with near tropical environments. The adult pest lays eggs on fresh fruits; usually before harvest of susceptible hosts such as Ananas sativa, Mangifera indica, Psidum guajava, avocadoes and other related fruits. The emergent larvae feed and develop inside the fruit depending on the host. The consequence of introduction and entry potential through imported fresh fruits are high. The outbreak risks based on climatic conditions marching the pest etiology and host biotics as well as pest survival between crops are also rated very high. Outbreak risks can however be greatly reduced to the level meeting the phytosanitary protection of the importing country if appropriate mitigation such as quality assurance and sorting, storehouse and pre-shipment treatment with environmentally safe pyrethroids insecticide and single point mitigation by gamma irradiation at the port of exit are applied.

Keywords:*fruit flies, Bactrocera invadens, phytosanitary protection, phytosanitary risks,*

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Occurrence and abundance of the insect pests associated with *Amaranthus* species in Ibadan Southwest Nigeria

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Survey of insects associated with the Amaranthus species was carried out in the Faculty of Agriculture and Forestry Plot beside PYTP Plot, University of Ibadan, Ibadan, Southwest Nigeria using quadrat. Three Amaranthus species: A. cruentus, A.blitum and A. hybridus were arranged in Randomized Complete Block Design and evaluated for abundance and diversity of associated insects using standard field evaluation procedures in the wet and dry seasons of 2009 and 2010. Data collected were analysed using Shannon (H) and Simpson (D) diversity indices and Evenness (E) respectively. A total of 59 insect species from 29 families and 12 orders comprising 31 defoliators, 16 minor pests, 12 predators and one (1) parasitoids were encountered on the Amaranthus species. The most abundant species in the wet season was Hymenia recurvalis (2916.8±138.83) followed by Hypolixus truncatulus (2262.7±94.10) and Lixus truncatulus (2088.7±36.37). In the dry season, similar trend occurred, Hymenia recurvalis (2311.5±32.46) followed by Clavigralla tomentosicollis (1456.3±17.77) and Lixus truncatulus (1142.3±25.58) was the most abundant species. The average species evenness (E= 0.65; 0.59) and diversity (D= 0.96; 0.96) for wet and dry seasons respectively were very high. Therefore, the most important defoliator of Amaranthus species is Hymenia recurvalis. Infestation of Amaranthus by this pest was all the year round on wild Amaranthus (Amaranthus spinosus) surveyed at the study site.

Keywords:*Hymenia recurvalis, Amaranthus species, Diversity indices and Evenness*

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Screening and identification of resistance donor cultivar of *Amaranthus* sp. against *Hymenia recurvalis*

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Beetworm Moth (BWM), Hymenia recurvalis is a foliage feeder that causes significant yield loss of Amaranthus in Nigeria. Nine Amaranthus cultivars were evaluated for resistance against Hymenia recurvalis in the screen-house and on the field using standard procedures in the wet and dry seasons of 2009 and 2010. Also, feeding preference of the BWM for different phonological stages of Amaranthus' leaves (1 to 6weeks) was evaluated using choice and no-choice bioassays. Resistance donor cultivar of the nine Amaranthus accessions against Hymenia recurvalis infestation was evaluated. However, best four accessions were assessed for antibiosis using standard procedures. Data on leaf area damage (LARD) and number of Hymenia recurvalis per plant were collected and analyzed using ANOVA ($p < 0.05$) and descriptive statistics and thereafter rated using standard visual rating scale to identify resistant donor. The most resistant Amaranthus species to H. recurvalis was A. hybridus (LARD= 12.47 ± 0.03; 0.3insects/plant) followed by A. blitum (LARD= 19.05 ± 0.05; 0.7insects/plant).

Keywords:*Beetworm Moth (BWM), Hymenia recurvalis, Amaranthus sp., Leaf Damage Area (LARD), Resistance donor.*

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Effectiveness of four protectants against the weevil, *Balanogastris kolae* (Coleoptera: Curculionidae) on kolantus, *Cola nitida* in storage

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Gammalin-20, though banned, is still widely used in the storage of Kolanuts in Nigeria. The effectiveness of Spintor (Spinozad), a naturally derived insecticide was compared with Gammalin- 20 and other protectants used in the storage of kolanuts against the weevil, Balanogastris kolae in Lagos and Osun States, Nigeria. Adult mortality was significantly highest in Spintor dust and Gammalin 20, higher in Alum and 'Omidun' and least in nuts treated with Phostoxin and Alum. Feeding marks, % damaged and % weight loss increased with days after treatment (DAT). The least damage was recorded at 90DAT in kolanut treated with Spintor at 2g/kg but not significantly different from treatments with Spintor (1g/kg) and Gammalin-20 (0.5ml/l). Spintor dust (1and 2g/kg) and Gammalin 20 (0.5ml/L), were relatively effective in protecting kolanuts from Balanogastris kolae infestations for 90DAT. Alum+Omidun was effective for 60DAT while Phostoxin was effective only for 30DAT but Alum+water and water (control) were not effective in the control of B. kolae infestation on kolanuts. The treatments had no effect on weight of the rats, Rattus rattus, as there was no significant difference ($p < 0.05$) between the weight of the rats fed on treated feed and the control. Histopathological tests on the liver of rats showed no disruptions in the hepatic lobes of rats fed with various treatments except on rats fed with G- 20 that had lesions in the liver. Results indicate that Spintor dust can be used as an alternative to Gammalin-20.

Keywords:*Kola nitida, Balanogastris kolae, Spintor, Gammalin20, Phostoxin, Alum, "Omidun," damage, feed*

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Effect of pollination by 3 species of pollinators, *Apis mellifera, Bombus terrestris, Osmia cornifrons* in the blueberry field

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To improvement quality of the blueberry fruit, 3 species of bee, Bombus terrestris, Apis mellifera and Osmia cornifrons were used for pollination in blueberry field in Pyeongtaek province. This study was also conducted using 3 kinds of methods for pollination effect, natural condition, net-house and control method. Blooming time of blueberry shows always during the May, foraging behavior of bees used in this examination showed different tendency each other. Especially pollinating time of B. terrestris was 2.77sec and transfer time between flower was 2.29sec. Rate of blueberry fruit setting pollinated with B. terrestris, A. mellifera, O. cornifrons were showed 87.7%, 89.6%, 66.5% respectively. And, rate of fruit setting of the untreated control and the control(natural condition pollination) were 15.4% and 80.3%. Quality of fruit pollinated with B. terrestris and A. mellifera showed higher than O. cornifrons in natural field condition in fruit weight. And, the rate of blueberry fruit setting pollinated with B. terrestris, A. mellifera, O. cornifrons were showed 87.7%, 89.6%, 66.5% respectively. And, rate of fruit setting of the untreated control and the control(natural condition pollination) were 15.4% and 80.3%. The rate of productivity increase obtained using the B. terrestris was 9%~17% greater than that obtained using natural pollination.

Keywords:*pollination, pollinators, Apis mellifera, Bombus terrestris, Osmia cornifrons, blueberry*

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An ecotoxicoproteomic approach (SELDI-TOF MS) to mixture toxicity of trace metals on eggs of mayfly, *Ephemera orientalis* (Ephemeridae, Ephemeroptera)

Hyoung-ho Mo[1], Yongeun Kim[2], Myung-seob Kim[3], Yun-sik Lee[4], Sung-Eun Lee[5], Key-Il Shin[6], Kijong Cho[7]

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Proteomics provide potential in the discovery of new sensitive biomarkers for environmental pollution. We utilized SELDI-TOF MS (surface enhanced laser desorption/ionization time-of-flight mass spectrometry) to analyze the proteomic profile of mayfly eggs (Ephemera orientalis) exposed to triple mixture of trace metals (lead, mercury and copper). In the previous experiment the HC10s (10% egg hatching inhibiting concentration) of each trace metal were determined by 2 week egg hatching test. In the three-component-mixture test, each metal ranged 1/6 to 1 of HC10, and the mixture design was augmenting simplex-lattice design. The protein peak patterns of E. orientalis eggs exposed to triple mixtures of trace metals were analyzed and compared using ProteinChip® Data Manager software (version 3.0, Bio-Rad, USA). This work could be the basics to develop the novel biomarker for aquatic ecological monitoring.

Keywords:*ecotoxicology, proteomics, Proteinchip®, water quality*

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Quantification and varietal variation of anthocyanin pigments, free sugars and fatty acids in mulberry fruits (*Morus alba* L.)

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This study was carried out to evaluate the components and content of anthocyanin pigments, free sugars, and fatty acids in mulberry fruits. The first, cyanidin-3-glucoside (C3G) of anthocyanin pigments in the mulberry fruits was separated and quantified by HPLC system using a Nova-Pack C18 column. Among the 38 tested accessions, one variety and thirty-four strains showed only C3G peak with 0.57 ~ 1.15 % contents, whereas C3G pigment was not detected in three accessions. The second, fructose and glucose were identified as the major free sugars. However, sucrose and maltose were not detected in this study. The mean contents of fructose and glucose were 4.49 ± 1.06 % DW, 4.62 ± 1.08 % DW, respectively. The third, palmitic acid, stearic acid, oleic acid and linoleic acid were detected as a major fatty acid in mulberry seed. Their rates of composition were 8.8±3.3, 4.2±1.6, 5.8±2.3, and 81.2±6.9(%), respectively. The unsaturated fatty acids such as oleic acid and linoleic acid were abundant in mulberry seed. From the above results, we conformed that mulberry fruits are of great value as functional food resources as well as fruits themselves of crop increasing income for farmers.

Keywords:*Morus alba L., mulberry fruit, anthocyanin pigments, free sugars, fatty acid, functional food resources*

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Effects of diamondback moth resistant transgenic cabbage (*Brassica oleracea*) on arthropod community structure

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The insect resistant transgenic crops may have an adverse effect on ecosystems because they may affect not only target species but also non-target species. We assessed the effects of diamondback moth resistant transgenic cabbage (Brassica oleracea) expressing insecticidal Cry1Ac toxin on arthropod community structure. The arthropod species on two transgenic cabbage lines (C-24 and C-30) and parental non-transgenic line (AD-126) were monitored in an isolated field located in Cheongwon in South Korea every week from 3 June to 15 July in 2011. We compared the structures of arthropod community using NMDS (non-metric multidimensional scaling) and ANOSIM (analysis of similarities). Although we found that abundance of Plutella xylostella was significantly different among cabbage lines, the result from NMDS and ANOSIM showed that there was no significant difference in arthropod community structures between transgenic lines of cabbage.

Keywords:*Arthropod, Brassica oleracea, community structure, Cry1Ac, transgenic*

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Fine structural analysis on the spinning nozzles for the viscid silks in the spider *Nephila clavata* (Araneae: Nephilidae)

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The fine structural characteristics of the spinning nozzles for capture thread production in the golden orb-web spider Nephila clavata were analyzed using scanning and transmission electron microscopes. Sticky and gluey capture threads of the web are originated from the silks of a pair of flagelliform glands and two pairs of aggregate glands. They supply precursors of the secretory silks to a pair of characteristic 'triad' spinning units on the posterior spinnerets. The aggregate gland is composed of large and multi-lobed secretory region and thick excretory duct surrounded by large irregular nodules. The nodules contain numerous mitochondria and glycogen particles within their cytoplasm and they are surrounded by the same sheath of thin connective tissue. The excretory duct of this gland basically consists of three superposed types of cells which are inner columnar epithelium, nodule forming cells and outer connectives. Secretory region of the aggregate gland which produces water-soluble components of the capture thread comprises discrete secretory vesicles and extensive rough endoplasmic reticulum.

Keywords:*Capture thread, microstructure, viscid silk, spider*

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Microstructural analysis of the mouthparts in the bark beetle *Ips acuminatus*, and their implication as external carriers of pathogens

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[1]Dankook University, Republic of Korea, [2]Dankook University, Republic of Korea, [3]Korea Forest Research Institute, Republic of Korea

The bark beetle Ips acuminatus Gyllenhal (Coleoptera: Curculionidae: Scolytinae) has been reported as a common group of bark beetle that infest and damage pine and spruce trees. These beetles are mostly plant feeders and are economically important pests of crops. Since the adult beetles chew the sapwood to create tunnels called galleries, they have developed effective drilling mouthparts enough to make galleries directly into the heartwood of the tree. The mouthparts of this bark beetle exhibit typical morphology of mycophagous coleopteran beetles and have those characteristics of chewing mouthparts that can excavate galleries in the hardwood. Here, we have analysed the fine structural aspects of its functional mouthpart using the field emission scanning electron microscopy (FESEM) for the purpose of understanding the mechano-dynamic characteristics and further biomimetic approaches. In addition, we also demonstrate their implication ability to act as external carriers of pathogens as a part of research for controlling this pest animal.

Keywords:*Bark beetle, microstructure, mouthpart, pathogen*

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Two generations of the European corn borer in Slovakia

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European corn borer (Ostrinia nubilalis) population in Gab?ikovo (47°53´59´´N; 17°37´6´´E), Slovakia, was examined for the incidence of its second generation in 2010. The first egg clusters of the second generation were found on maize leaves on August 23 with consecutive ear damage firsty found on September 3. At the beginning of October, more than 50 % of the maize plants were infected by the larvae of the second generation. On October 7, together 660 larvae were collected from the maize cobs to record their developmental stage. Climatic conditions in 2005-2010 are discussed and compared with climatic conditions in 1990´s when, for the first time, a partial second generation occurred in Slovakia. The results show that degree-days accumulation (base 10°C) from the beginning of the year to the date of the first egg clusters in June and for the whole year did not influence the development of the second generation. Higher temperatures in the period from the beginning of July to the end of October in 2005-2010 seem to be responsible for the development of the second generation. Furthermore, changing climatic conditions (high temperatures in summer and autumn) probably led to the shift of bivoltine population northwards to historically univoltine habitats.

Keywords:*Ostrinia nubilalis, European corn borer, voltinism, climatic conditions, diapause*

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A novel mosquito feeding system for routine blood-feeding

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Vector-borne disease research often requires maintenance of laboratory colonies of vectors. Blood feeding of mosquitoes is thus required for the maintenance of these colonies. Due to the increasing awareness on animal welfare and stringency in regulations governing the scientific use of animals for research, coupled with the inconvenience of using live animals as blood hosts, we developed a simple and user friendly blood feeding system for artificial feeding the mosquitoes The system consisted of a collagen membrane casing filled with specific pathogen free (SPF) mini-pig blood, which is warmed by a simple in-house designed heating device. Blood feeding rate, fecundity, survival rate and hatchability of Ae. aegypti and Ae. albopictus colonies maintained by the feeding system were compared with those raised by conventional guinea pig feeding method. Aedes aegypti, displayed a significant difference in the feeding rate when offered blood meal using the membrane feeding (85.3%) and the guinea pig feeding (96.2%) methods (P=0.012). Though the feeding rate was reduced, the level was acceptable for maintenance of laboratory colonies. There was no significant difference in the fecundity (P=0.556), survival rate (P=0.715), and hatchability (P=0.932) between the two methods. For Ae. albopictus, the two feeding methods yielded no significant difference for the three parameters (fecundity, survival rate and hatchability=0.887, 0.580 and 0.564, respectively). Hence, we conclude that this simple collagen based membrane blood feeding system can be used for routine colonization of laboratory strains of Ae. aegypti and Ae. albopictus.

Keywords:*membrane feeding, Aedes aegypti, Aedes aegypti*

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Dispersal patterns of pine wilt disease at the newly infested areas

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Pine wilt disease (PWD) caused by the pine wood nematode (PWN), Bursaphelenchus xylophilus, is a serious pest for pine trees in many countries including South Korea, Japan, China, Taiwan and Portugal. The Japanese pine sawyer and long-horned beetle, M. saltuarius, are vectors of PWN in South Korea, causing the disease dispersal. In Korea, after the first occurrence of PWD in Busan in 1988, PWN had expanded its infestation areas in Korean nationwide scale. Better understanding on the dispersal patterns of the disease is essential for installing the control strategy of PWD. In this study, we evaluated the dispersal patterns of PWD at the newly infested areas using data monitored from 1998-2004. Both area and the number of patches (infested area), dispersal patterns, and dispersal distance were measured at two different spatial scales (stand and region) using ArcGIS. We defined four different dispersal types in patches: the similar patch shape with the previous year, movement of patches, dispersal only one direction, and dispersal of multiple directions. The areas and the number of patches infested were increased as a function of year after the first infestation. Annual dispersal distance increased with the time progress. Maximum dispersal distance in each year increased, while minimum dispersal distance decreased, resulting from increase of the number of patched infested by PWN. We discussed the efficiency of control activities for different dispersal patterns at the nearly infested areas.

Keywords:*pine wilt disease, pine wood nematode, dispersal pattern*

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Comparative genomic analysis of the UDP-glycosyltransferase multigene family in insects

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UDP-glycosyltransferases (UGT) catalyze the conjugation of a range of diverse small lipophilic compounds with sugars to produce glycosides, playing an important role in the detoxification of xenobiotics and in the regulation of endobiotics in insects. Recent progress in genome sequencing has enabled an assessment of the extent of the UGT multigene family in insects. Here we report over 310 putative UGT genes identified from genomic databases of ten different insect species including four lepidopteran species. Phylogenetic analysis of the insect UGTs showed Order-specific gene diversification and inter-species conservation of this multigene family. Only one family (UGT50) is found in all insect species surveyed (except the pea aphid) and may be homologous to mammalian UGT8. Three families (UGT31, UGT32, and UGT305) related to Lepidopteran UGTs are unique to baculoviruses. A lepidopteran sub-tree constructed with 40 H. armigera UGTs and 44 Bombyx mori UGTs revealed that lineage-specific expansions of some families in both species appear to be driven by diversification in the N-terminal substrate binding domain, increasing the range of compounds that could be detoxified or regulated by glycosylation. Many UGTs were expressed in fat body, midgut, and Malpighian tubules, consistent with functions in detoxification, and some were expressed in antennae, suggesting a role in pheromone deactivation. These findings from this comparative study of two lepidopteran UGTs as well as other insects reveal a diversity of this gene family and show the magnitude of the task ahead, to determine biochemical function and physiological relevance of each UGT enzyme.

Keywords:*UDP-glycosyltransferase, genomics, multigene family, detoxification*

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OCH-group (Odonata-Coleoptera-Hemiptera-group), the representative aquatic insect group in the Upo Wetland, Korea

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The Upo Wetland is the largest riverine wetland in Korea. It was formed about 1 million years ago by the flooding effect of the Nakdong River. This study aimed at an investigation of representative aquatic insect group in the wetland. Field survey was conducted in the spring of 2012, and related literatures on Korean wetland fauna were reviewed. Hand nets were used to collect aquatic insects and other benthic macroinvertebrates. Collected specimens were identified in the laboratory using available key books and references. As a result, 135 species, 105 genera, 59 families, 16 orders, 7 classes, and 3 phyla of benthic macroinvertebrates were identified in the whole area of the Upo Wetland. The Odonata-Coleoptera-Hemiptera-group (OCH-group) was known to be the representative aquatic insect group in the Upo Wetland. It showed > 50% of total species richness, even higher than that of non-Insecta group (33.75%). As analyzed with other reference wetlands in Korea, the OCH-group was shown to be the representative wetland aquatic insect group demonstrating > 50% of whole benthic macroinvertibrate communities (Deokso 57.6%, Mungyeong 66.7%, Dunchon 61.5%, Jingwannae-dong 66.7%, and Gokseong 58.6% wetlands). On the other hand, the OCH-group composition of the Topyeong Stream (29.7%), a lotic section of the Upo Wetland, Gapyeong Stream (2.8%), a typical mountain stream in Korea, and Andong Lake (19.6%), a large dam-lake in Korea, were relatively low. From this result, it is suggested that the OCH-group composition is the key characteristics in determining habitat conditions of wetland ecosystems in Korea.

Keywords:*aquatic insects, OCH-group, Upo Wetland, biodiversity, Korea*

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Studies on taxonomy, bology and pest status of Indian biotypes of *Acanthoscelides macrophthalmus*(schaeffer) (Coleoptera : Bruchidae)

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A large number of non-native insect species invading new areas either accidentally or through man mediated trading consignments. Similarly many pulse beetles (bruchids) have crossed the geographical boundaries and becoming cosmopolitan in distribution through human-mediated migrations or accidently, thus posing major pest problem worldwide particularly in the absence of their natural enemies. A leucaena bruchid beetle, Acanthoscelides macrophthalmus (Schaeffer) (Coleoptera: Bruchidae) native to Central and Southern America has somehow crossed the geographical boundaries and widely spread in different states of the India and found infesting seeds of Leucaena leucocephala (Lamark) de Wit plant almost throughout the subcontinent. Acanthoscelides Schilsky (1905) is one of the largest genus of the tribe Acanthoscelidini containing more than 340 species globally and the species, macrophthalmus was described by Schaeffer in 1907 and placed under genus Bruchus but Johnson in 1968 rightly put the species under genus Acanthoscelides. The congeneric species, A. pallidipennis reported from China, Korea and Japan and A. obtectus (Say) has been recently reported from India. Studies of Indian bruchids carried out so far did not reveal the complete taxonomy, biology and pest status of this species except a mere reference of its presence from the pods of Albizia lebbeck (L.) Benth from southern part of the country. However, phyotosanitary risk of thirteen bruchid species in legumes bulk imports has been alarmed by National Quarantine Division (NQD) and hidden infestation instances of different species belonging to Acanthoscelides, Bruchidius and Caryedon genera and their X-ray screening immediately on arrival in Indian Territory was advocated.

Keywords:*Taxonomy, biology, Acanthoscelides macrophthalmus, Leucaena leucocephala, pest status.*

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Plant-based insect repellent: is that an appropriate strategy to malaria burden in Ethiopia

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Malaria is a major public health issue in the tropical and sub-tropical countries of the world, particularly in Ethiopia. The majority of the malarial victims are the adults who live in the resource-constrained remote areas, children and the expectant mother. Personal protection measures are the key interventions to prevent arthropod-borne diseases in order to reduce the disease transmission. Although insecticide treated nets (ITNs) and indoor residual spraying (IRS) are effective in minimizing the vector-borne disease burden, it may not be effective when the malaria vectors are exophilic and exophagic. Furthermore, in East Africa particularly in Ethiopia, Anopheles gambiae complex's biting activity tends to start in the early evening hours. In this view point, repellents could play the pivotal role. However synthetic repellents are expensive, can cause allergic reactions and a few of them require electricity. In this context, plant-based insect repellents could be extremely useful and helpful. Although usage of plant-based insect repellents is a part of the Ethiopian tradition and culture, there are many issues which need to be addressed to minimize the malaria burden in Ethiopia. Even though plant-based insect repellents cannot be employed as a frontline vector control tool, it could serve as an appropriate/supplementary tool in combination with the existing malaria control interventions like ITNs and IRS.

Keywords:*Malaria, Plant-based Insect Repellents, ITNs, IRS, Ethiopia*

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Morphometrics and aspects of Bio-ecology of *Ephestia cautella* (Lepidoptera : Pyralidae) on stored date fruit (*Phoenix dactylifera*) and hermetic control technique

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Rate of fecundity, life cycle, morphometic characteristics of Ephestia cautella and control were investigated. These were done on sterilized dried date (Phoenix dactylifera) as substrate. Daily fecundity of 17 eggs was recorded, while a total of 1117 eggs were laid by 15 adults female E.cautella within 6 days of oviposition in perforated date. The life cycle from egg to adult took 37 ± 1.92 days, under a temperature and relative humidity of 32.5 ± 1 0C and 70 ± 5% respectively. First filial generation (F1) of 1, 217 adults emerged after 30 days of life cycle study, with sex percentage ratio of 54.7%: 45.3%, males and females respectively. The morphometric characteristics of E.cautella revealed that female and male adult mean total body length ranges between 8.2 - 10.0 mm and 7.9 - 8.9 mm respectively. The control set up on larval stage using varied polythen bag layers of three, two, and one layer with thickness of 0.056mm for hermitic control treatment confirmed that three bags layers was the best treatment. Recorded percentage mortality values of 74.20%, 19.36% and 0.26% were obtained respectively from the set up.

Keywords:*E.cautella, Phoenix dactylifera, Fecundity, Life cycle, Morphometric, Hermitic control*

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Effects of gibberellic acid (GA3) on hemocyte numbers, morphology and mitotic indices of *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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The impacts of different doses of the plant growth regulator, gibberellic acid (GA3) in diet on the number of total and differential hemocytes, and mitotic indices were investigated using Galleria mellonella L. (Lepidoptera: Pyralidae) larvae. To investigate the effects of different doses of GA3 (50 to 5,000 ppm) on the number of circulating hemocytes, last instars were used and hemolymph suspension diluted in anticoagulant solution were applied to an improved Neubauer Hemocytometer. Different and mitotic hemocyte counts were assayed by Giemsa dye method. Total (THC) and differential hemocyte counts (DHC) were assayed under a Leica DM6000 B microscope with bright field and phase contrast optics. Exposure of G. mellonella to a diet containing GA3 resulted in a remarkable increase in the number of circulating hemocytes at doses <1,000 ppm with respect to untreated larvae whereas GA3 application had no effect on the number of different hemocyte types. The percentage of mitotic index also increased at 5,000 ppm. We suggest that lower doses of GA3 may induce hormesis effect that is characterized as low dose stimulation of potentially toxic substances at sublethal concentrations. Consequently, increasing amount of GA3 in diet of larvae negatively influenced the cellular immune defence of insects.

Keywords:*Galleria mellonella, hemocyte, Gibberellic acid*

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Determination of diazinon residues in grape and sour grape

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Organophosphorous, markably diazinon, are still used extensively in vineyards in Iran in order to suppress pests such as the European grapevine moth (Lobesia botrana). Considering the fact that grape is mostly consumed fresh in Iran and verjuce, a product which is made from fresh unripe green grapes (sour grapes), is widely used in oriental cuisine specially in Persian cooking. Residue of this pesticide in table grapes and sour grapes can pose harmful effects on the consumer's health. The residues and dissipation pattern of diazinon on grape and sour grape was studied under field conditions. Diazinon was applied to a local vineyard variety at recommended dose (600 mg a.i.L-1) with three intervals. Representative samples were taken at 0, 1, 2, 5, 8 and 12 days after the second spray (sour grape stage) and up to 32 days after the last spray (grape stage). Extraction was done by shaking the homogenized samples with a mixture of methanol: acetonitrile:deionized water (1:1:1 v/v/v). Solid phase extraction was used for clean up and quantification analysis of the residues was done using a gas chromatograph apparatus equipped with NPD detector. Results revealed dissipation of diazinon in sour grape and grape followed first-order kinetic and the equations were C0= 4.4631e-0.46t and 8.5844e-0.21t with half lives of 1.5 and 3.3 days respectively. Diazinon residues in sour grape and grape reached below the Maximum Residue Level (MRL) of 0.5 mg.kg-1 after 5 and 15 days respectively.

Keywords:*Organophosphorus, Grape, Sour grape, Maximum Residue Level, First-order kinetics.*

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Larvicidal activity of essential oils from *Thymus kotschyanus* and *Mentha longifolia* against *Tribolium castaneum*

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This study was conducted to establish the larvicidal activity of essential oils from Thymus kotschyanus Boiss and Hohen and Mentha longifolia L. against one-day old larvae (one DOL) and 10-days old larvae (10 DOL) of Tribolium castaneum (Herbst). The flowering dried parts of the plants hydrodistillated by a clevenger-type apparatus for 3 h. Different concentrations from 415.38 to 723.08 µL/L air of T. kotschyanus and from 0.6 to 1.6 µL/L air of M. longifolia were used against one DOL and the concentrations from 400 to 784.62 µL/L air of T. kotschyanus and from 0.3 to 1.3 µL/L air of M. longifolia were used against 10 DOL to determine the larvicidal activity of the essential oils. After 24 hours from commencement of exposure, larval mortality was recorded. Experiment was carried out at 26±1 C°, 65±5 RH% in dark condition and in five replications with 20 larvae in each replication. Data were analyzed by Probit analysis using the SAS program. LC50 values of the T. kotschyanus and M. longifolia were 543.49 and 10.15 µL/L air against one DOL, and 580.70 and 15.28 µL/L air respectively against 10 DOL of T. castaneum. The results show that M. longifolia was significantly exhibited much stronger activity than T. kotschyanus and usually one DOL was more susceptible to the essential oils.

Keywords:*Mentha longifolia, Thymus kotschyanus, Tribolium castaneum, Larvicide*

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Population density of *Edwardsiana rosae* L. and *Ardis brunniventris* Hartig on different genotypes of *Rosa damacena* in West-Azarbaijan province, Iran

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Rosa damascena Mill is one of the most important medicinal plants that use its ooze, distillates and oil. Various pests cause damage to ornamental and medicinal plant, annually. Because of unfavorable effects of pesticides in products of R. damascena and environmental ecosystem, using a combination of other control methods should be considered. Evaluate the population and damage of the pests in the different genotypes of Damask rose would be a noticeable step in this regard. During the 2010-2011 years, contamination of 36 genotypes of R. damascena was evaluated to important pest in the Urmia, West-Azarbaijan province. Field studies were carried out in damask rose in Saatlo Research Station, Urmia. Field samplings were carried out using different methods for each pest. Analyze of variance revealed a significant difference between genotypes. The lowest density of winter eggs of Edwardsiana rosae L. were observed on 1, 12, 16, 22, 10 and 14 genotypes originated from East-Azerbaijan, Zanjan, Fars (No.1), Kohkluye, Gom and Semnan (No. 2), but the highest prefer to spawning egg were observed on 39, 32, 35, 6, 17, 25 and 21 genotypes originated from Esfahan (No.7), Yazd (No. 2), Esfahan (No. 2), Ilam, Fars (No. 2), Gilan and Kermanshah The lowest contamination to Ardis brunniventris Hartig has been observed on 29, 13, 14, 17, 18, 30 and 7 genotypes originated from Hormzgan, Semnan (No.1), Semnan (No. 2), Fars (No. 2), Gazvin, Hamadan and Tehran.

Keywords:*Edwardsiana rosae, Genotype, damask rose, Ardis brunniventris, Iran.*

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Biological controllers of *Opsiphanes cassina* (Lepidopterous: Brassolidae) in plantations of African oil palm, Venezuela, South America

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The African oil palm is attacked by insects pest as Opsiphanes cassina, fact that reduces the productivity of the plantations. The aim of the study was to determine the relation between these insects pest, their parasites and the plants weeds present in oil palm plantation, Zulia state, west of Venezuela. The immature specimens of O. cassina were captured in field and supported in cages, where the parasites emerged of the pustules was captured. Also they were captured parasites in the weeds Senna reticulata, Solanum hirtum and Solanum sp. was calculated the index of diversity of Shannon-Weaver (H') and the parasites were identified. Thirty species of insects parasites were captured; 5 parasitized the individuals of O. cassina: Conura sp1, Conura sp2 and Conura sp3, Brachymeria sp and Cotesia sp. The biological diversity of the community of insects parasites was of: 1,22, 0,25 and 0,24 in Senna reticulata, Solanum hirtum and Solanum sp, respectively. The 5 species of parasites were always present in Senna reticulata, with exception Conura sp3, that also appeared in Solanum hirtum and in Solanum sp. Was not find statistically significant differences ($p<0,05$) between the populations of 5 species of parasites and the studied weeds. One found a relation of parasitism between 5 species of wasps and O. cassina. These species of parasites are associated with the present weeds in the plantations of oil palm, which might be used in the managing of this insects pest.

Keywords:*IMP, oil palm*

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Predatory Efficiency of *Chrysoperla zastrowi* sillemi (Esben-Peterson) and *Cryptolaemus montrouzieri* Mulsant on *Paracoccus marginatus* and *Phenacoccus solenopsis* infesting Sunflower

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Predatory potential of Chrysoperla zastrowi sillemi and Cryptolaemus montrouzieri on Paracoccus marginatus and Phenacoccus solenopsis infesting sunflower was studied. Results revealed that grubs of C. zastrowi sillemi fed 6.2 ± 1.1, 448.0 ± 76.4 and 68.8 ± 9.5 ovisacs, nymphs and adults of P. marginatus and 426.2 ± 9.2, 150.7 ± 3.3, 294 ± 4.0 first instar, second instar and third instars of P. solenopsis to complete their life stages. Similarly, grubs of C. montrouzieri fed 7.6 ± 0.7, 153.6 ± 23.8 and 62.8 ± 11.6 ovisacs, nymphs and adults of P. marginatus and 92.2 ± 8.5, 57.4 ± 5.8 and 22.2 ± 4.7 first, second and third instars of P. solenopsis, respectively to complete their life stages. Third and fourth instar were voracious and consumed 2.2 ± 0.4, 43.6 ± 2.0 and 23.2 ± 3.0 and 2.8 ± 0.4, 69.6 ± 4.6 and 27.6 ± 4.6 ovisacs, nymphs and adults of P. marginatus and 20.4 ± 2.0, 16.8 ± 1.6 and 9.2 ± 1.7 and 51.8 ± 3.2, 32.8 ± 2.7 and 11.6 ± 2.1 first, second and third instars of P. solenopsis, respectively. Adult C. montrouzieri consumed on an average 5.2 ± 0.8, 101.2 ± 13.5 and 35.4 ± 8.6 ovisacs, nymphs and adults of P. marginatus and 118.6 ± 10.4, 198.6 ± 15.0 and 42.2 ± 4.7 first, second and third instars of P. solenopsis, respectively. It is concluded that C. zastrowi sillemi and C. montrouzieri can be effectively employed in the management of sunflower mealybugs.

Keywords:*Chrysoperla zastrowi, Cryptolaemus montrouzieri, Paracoccus marginatus, Phenacoccus solenopsis*

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Classical biological control of papaya mealybug, *Paracoccus marginatus* (Williams and Granara de Willink) using Acerophagus papayae (Noyes and Schauff) in Tamil Nadu, India

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Paracoccus marginatus is a polyphagous pest recorded from 135 host plants. It was recorded in papaya for the first time in Coimbatore, Tamil Nadu, India during July, 2008. Classical biological control was identified as an important component in the management of P. marginatus. Field efficacy of A. papayae on P. marginatus showed significant variations among pre and post-release counts. Population of P. marginatus ranged from 17 to 59 per 2.5 cm2 leaf area before release of parasitoid. Thirty days after release (DAR), the population of mealybug ranged from 8 to 23 per 2.5 cm2 leaf area corresponding to a reduction of 38.9 to 70.6 per cent. In unreleased field, the mealybug population was 28 at one month after release. At 60 DAR, significant reduction in the mealybug population was observed in all locations. Mealybug population ranged from 0 to 6 per 2.5 cm2 leaf area and reduction in population ranged from 72.2 to 100 per cent. Data on recovery of A. papayae revealed that maximum recovery of 81.3 per cent was recorded at 30 DAR. This confirms the establishment of A. papayae at all the experimental fields where the parasitoids were released. In unreleased field, 9.6 per cent recovery was obtained at 30 DAR. At 45 DAR, 68.7 to 89.6 per cent recovery was observed at different locations. In unreleased field, 16.7 per cent recovery was obtained at 45 DAR. The results revealed the establishment and recovery of A. papayae in all the fields providing successful control of P. marginatus.

Keywords:*Classical biological control, Paracoccus marginatus, Acerophagus papayae, papaya, field recovery*

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Preliminary study of *Oligonychus afrasiaticus* predators in Manoojan (south of Iran)

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During recent years, Oligonychus afrasiaticus (McGregor, 1939) has been one of the major pests of date palms in Iran (Kamali et al., 2001). To investigate the natural enemies of this pest, a faunistic survey of predatory mites was done during 2010-2011 in Manoojan. Pest colonies on the green fruits was collected and sampled. A total of six species was collected from which, Molothrognathus mehrnejadi and Neoseiulus barkeri were the most abundant species respectively. Collected predatory mite species are as follows: Raphignathidae Raphignathus hecmatanaensis Kanjani & Ueckermann, 2003 Bdellidae Spinobdella Cornini Baker & Balock, 1962 Phytoseidae Neoseiulus barkeri Huges, 1948 Stigmaeidae Cheyletostigmaeus sp. Caligonellidae Molothrognathus mehrnejadi Liang & Zhang 1997 Cunaxidae Lupaeus Lectus Catro & Den Heder,2009 Kamali, K., Ostovan, H. & Atamehr, A. (2001) A catalog of Mites & Ticks (Acari) of Iran. Islamic Azad University Scientific Publication Center. Tehran, 127 pp. McGregor, E.A. (1939). The specific identity of the American date mite; description of two new species of Paratetranychus. Proceedings of the Entomological Society of Washington, 41: 247-256.

Keywords:*Tetranychidae, predator, Oligonychus afraciaticus, date palm, Iran*

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The report of some typical oriental water mites (Acari: Hydrachnidia) from another province in south of Iran (Manoojan)

Saeid arjmand[1], isa etemadi[2], Mahdieh Asadi[3]

[1]Professor. Hesabi institute of Manoojan, Manoojan, Iran, Iran, [2]vali-e-asr university of Rafsanjan, Iran, [3]Shahid Bahonar University of Kerman, Iran, Iran

A big part of Iran is placed in Palearctic region, but south of Iran is transitional between the Palaearctic, Oriental and Afrotropical region, and a hot belt in the south of Iran (Sistan va Baluchestan, Hormozgan) is often regarded as a part of the Oriental region. In a faunistic survey of water mites done in the south of Kerman province in 2011, eight families of water mites collected. One new genus and three species were described as new for science (Asadi et al., 2010) The most interesting point is that It seems that fauna of streams and pools near Manoojan is different from other part of Iran and contain typical Oriental elements. With regards to the previous work in kerman province and the faunal structure of this part of Iran (Asadi, 2010), it seems that this boundary is placed in Kerman province. More investigation will help us to detect this boundary in south of Iran. All of the species collected are as follows; oriental typical species are marked by asterisk. Hydrodroma reophila Cook, 1967 Sperchon (Hispidosperchon) papillosus Thor, 1901 Axonopsis (Hexaxonopsis) manoojani Pesic & Asadi, 2010 Axonopsis (Hexaxonopsis) iranica Pesic & Asadi, 2010 Limnesia kochi* Smit & Pesic, 2010 Limnesia (Tetralimnesia) pinguipalpis* Cook,1967 Neumania maharashtris* Cook,1967 Neumania indentata* Smit & Pesic, 2010 Hygrobates fluviatilis Stom,1768 Iranobates hesabii Pesic, Smit & Asadi, 2011 Atractides omanensis* Smit & Pesic, 2010 Nilotonia persica Pesic & Saboori, 2006 Oxus cf. angustipositus K. Viets, 1908 Asadi, M., Pesic, V. and Etemadi, I. (2010) A

Keywords:*Palearctic, water mite, Iran, Manoojan, Fauna*

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First report of the marine water mites *Pontarachna arabica* (Hydrachnidia : Pontarachnidae from Iran

Isa Etemadi[1], Mahdieh Asadi[2], Malihe Latifi[3]

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Pontarachnidae are one of the least know water mite families, Nothing is not know about their life cycle, hosts or food. They occur almost worldwide, but most species are found in subtropical or tropical areas. A few species have been found in freshwater, but all of these occur in estuaries or locations near the seas (Smit, 2008). During a sampling in the sea shores of Persian Gulf and Oman Sea in May 2012 this family collected and identified as Pontarachna arabica Pesic, Chatterjee & Ahmed Abada, 2008. This family and species reported for the first time from Iran and the male of this insect is under describing for the first time.

Keywords:*Acari, Iran, Pontarachna Arabica, water mite*

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Acetylcholinesterase insertional mutations in insecticide-resistant populations of Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say)

Maryam Malekmohammadi[1], Mir Jalil Hejazi[2], Mohammad Saeed Mossadegh[3], Hamid Galehdari[4], Mohammad Khanjani[5], Mohammad Taghi Goodarzi[6]

[1]Bu Ali Sina University, Iran, [2]University of Tabriz, Iran, [3]Shahid Chamran University, Iran, [4]Shahid Chamran University, Iran, [5]Bu Ali Sina University, Iran, [6]Hamedan University of Medical Sciences, Iran

Insecticide-resistant populations of Colorado potato beetle (CPB), with insensitive acetylcholinesterase (AChE) have recently been reported from commercial potato fields of Hamedan province in west of Iran. PCR-SSCP method was used to find sequence variation in the AChE gene. Based on the published nucleotide sequence information on AChE gene (GenBank L41180.1), five primer pairs were designed to amplify specific PCR products of 306 bp (first fragment: codons 24 through 142), 370 bp (second fragment: codons 141 through 261), 403 bp (third fragment: codons 248 through 381), 335 bp (fourth fragment: codons 376 through 486), and 335 bp (fifth fragment: codons 488 through 598), respectively. No specific PCR product of desirable size for either the fourth and fifth fragments could be obtained. The DNA amplification products were subjected to SSCP analysis to identify the DNA sequence variations between the susceptible strain and resistant populations. Alignment of the AChE gene sequences with the sequencing results revealed 25 single-base insertional mutations at nt 303, 305, 340, 605, 607, 764, 772, 855, 856, 891, 892, 897, 1084, 1099, 1113, 1137, 1147, 1178, 1195, 1246, 1247, 1248, and 1249 from the field populations of CPB, which may contribute to the AChE insensitivity. Further analysis on the RNA level is necessary for clarification and validation of this contention.

Keywords:*Colorado potato beetle, AChE gene, PCR-SSCP, insertional mutation*

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Acetylcholinesterase deletion mutations in insecticide-resistant populations of Colorado Potato Beetle, Leptinotarsa decemlineata (Say)

Maryam Malekmohammadi[1], Mir Jalil Hejazi[2], Mohammad Saeed Mossadegh[3], Hamid Galehdari[4], Mohammad Khanjani[5], Mohammad Taghi Goodarzi[6]

[1]Bu Ali Sina University, Iran, [2]University of Tabriz, Iran, [3]Shahid Chamran University, [4]Shahid Chamran University, Iran, [5]Bu Ali Sina University, Iran, [6]Hamedan University of Medical Sciences, Iran

Insecticide-resistant populations of Colorado potato beetle (CPB), with insensitive acetylcholinesterase (AChE) have recently been reported from commercial potato fields of Hamedan province in west of Iran. PCR-SSCP method was used to find sequence variation in the AChE gene. Based on the published nucleotide sequence information on AChE gene (GenBank L41180.1), five primer pairs were designed to amplify specific PCR products of 306 bp (first fragment: codons 24 through 142), 370 bp (second fragment: codons 141 through 261), 403 bp (third fragment: codons 248 through 381), 335 bp (fourth fragment: codons 376 through 486), and 335 bp (fifth fragment: codons 488 through 598), respectively. No specific PCR product of desirable size for either the fourth and fifth fragments could be obtained. The DNA amplification products were subjected to SSCP analysis to identify the DNA sequence variations between the susceptible strain and resistant populations. Alignment of the AChE gene sequences with the sequencing results revealed sixteen single-base deletions at nt 241, 487, 527, 535, 575, 860, 862, 884, 886, 892, 901, 1169, 1177, 1200, 1241, and 1243 from the field populations of CPB, which may contribute to the AChE insensitivity. Further analysis on the RNA level is necessary for clarification and validation of this contention.

Keywords:*Colorado potato beetle, AChE gene, PCR-SSCP, deletion, mutation*

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Others

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PGPR as biofertilizers: Their impacts on productivity of lentil and ckickpea and population incidence of associated insect fauna

Muhammad Dildar Gogi[1], Muhammad Ashfaq[2], Muhammad Jalal Arif[3], Muhammad Irfan Ashraf[4], Rashad Rasool Khan[5], Muhammad Rafiq Shahid[6]

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Beneficial rhizosphere microorganisms are generally classified into plant growth promoting microorganisms (PGPM) that directly promot the plant growth and biological control agents (BCA) that indirectly contribute to plant productivity through the control of plant pests. Some species of microorganisms including mycorrhiza, pseudomonas and nitrogen-fixing bacteria are considered as mediators of paramount importance for the determination of nature and type of interactions between plants and herbivores (Barbosa et al. 1991). These studies were carried out to investigate the impact of a two formulations/mixtures biofertilizers i.e., FOR-1 (consisting of three strains of plant growth promoting rhizobacteria (PGPR) i.e., J1, JH4 and B5) FOR-2 (consisting of four strains of plant growth promoting rhizobacteria (PGPR) i.e., LLR3, LC3, CB5 and LC4) on the productivity of two pulses (lentil and chickpea) and incidence of insect fauna (both beneficial and pest insects). The application of both formulations of PGPR induced significant effects on the productivity of both pulses intern of yield ($p<0.01$) and resulted in 2-3 times more yield than conventional application of synthetic fertilizers. Similarly, PGPR application also promoted the host plant resistance enhancing the nutrient availability and leaf contents of treated plants as compared to synthetic fertilizers. In PGPR treated plots, higher numbers of beneficial insect fauna and lower number of insect pest fauna was observed as compared to plots which were treated with synthetic fertilizers. The results emphasized the promotion of PGPR application in pulses agroecosystem. The regression and correlation results is also discussed.

Keywords:*Insect Biodiversity, PGPR, Pulses, Host-Plant Interaction*

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P7

Investigations on development stages of *Lucilia caesar* Linne, 1758 (Calliphoridae: Diptera) in terms of forensic Entomology

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Calliphoridae family larvae are important for forensic entomology. For that reason development biology of larvae are very important. Larvae and adult stage nutrition habits are different at members of that family which get holometabol metamorphosis. In generally Calliphoridae species lives on that dead or living in at larval stages. Species that lives on dead in at larval stage helps for ecological balance by easier to take to pieces and decay. On the other hand classes which lives on living in at larval stage causes "Myiasis" illness. Larvae that have the ability of take texture in to pieces very faster, uses in uncured bruise. This study make with samples taken by one stations in city Eskisehir in TURKEY. For continue that study putrid hepatic pieces had put at station and egg packets add together over that hepatic. Egg packet which are in Calliphoridae family and the time of larval stages to reach adult. At that study one base family inculudes one genus and one species Lucilia caesar Linne, 1758, had fasten down. It has seen that development of investigated with Lucilia Caesar at 15-19 days were finished.

Keywords:*Forensic Entomology, Calliphoridae, larvae, development biology*

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PS7TH402

Others

P7

Sustainable agriculture through a miracle plant of Pakistan

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Forests play a major role for economical and environmental benefits of man. Plants are valuable for us in many ways, besides protecting and improving the environment in' which we live, they control run off, check floods and soil erosion, improve soil fertility and help in reducing temperature and pollution. Regarding all the benefits associated with plants, Moringa tree is most suited to the people of Pakistan. It is considered one of the world's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial property. It is widely used as food and medicine for both human and other animals. Oil of Moringa retains the properties of use as biofuel, pesticide and natural oil. For evaluating the efficacy of Moringa plant, a study was conducted at Faisalabad and Moringa Leaf Extract was used as foliar application in wheat under field conditions. An increase of 10.73, 6.00, 10.70 and 4.00% was seen in 1000 grain weight, biological yield, grain yield and harvest index respectively, when crop was sprayed with MLE at tillering + jointing + booting + heading. MLE can also be used as priming agent in hybrid maize.

Keywords:*Moringa, Miracle plant, biodiesel*

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Others

P7

Using species of Ephemeroptera as saprobic indicator to determine water quality in Sultansuyu stream (Malatya, Turkey)

Caner Aydinli
Anadolu University, Turkey

1795 Ephemeroptera (Insecta) nymph samples from 7 localities in Sultansuyu Stream (Malatya) were collected and investigated. From these nymph samples, 14 species belonging to 10 genera in 6 families were determined. The species determined in the research area was also evaluated according to saprobic system and tried to determine water quality of Sultansuyu stream. When water quality evaluated according to dominance analysis at genus level, results did not show compliance with water quality observed in studying area. For this reason, water quality, were conducted with the species level. According to the results of this study, it is determined that 7th and 6th localities which were near to spring showed the characteristics of clear water (oligosaprobic), 5th and 4th localities showed the characteristics of little dirty (β-mesosaprobic) and the localities from 3rd to 1st showed the characteristics of dirty water (α-mesosaprobik).

Keywords:*Ephemeroptera, Saprobic system, Biotic Index, Biotic Indicator, Sultansuyu Stream,*

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Others

P7

Determination of diazin pesticide residue in rice by gas chromatography /mass spectrometer in Mazandaran Province in Iran

sohrab imani[1], Shahrzad Mohammadi[2], Ahmad reza Golmohammadi[3], Sina Imani[4]

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The application of pesticides is essential in effectively controlling pests and any inappropriate use of pesticides could cause residue problems. Rice is one of the most important food sources of people in the world. Chilo suppresalis is its key pest in Mazandaran province and Diazinon is the most common pesticide that is used against it. Due to the importance of this product & regarding the fact that it provides two-third of people's daily calories in the Asian region this study is conducted for the awareness of Marketed rice of contamination of Diazinon residue in this area which has the first rank in the amount of cultivation of desired product .Thirty two samples were randomly collected from the Mazandaran province farms and stores. In the laboratory, each sample was extracted with using Solid Phase Extraction method and the final extracts were injected into Gas Chromatography /Mass Spectrometer (GC/MS) system. Obtained data were compared with WHO/FAO Codex Alimentary Commission by T-test. Results showed pesticide contamination in all cases and their volumes were from 0.007 to 2.26 ppm .In 13 cases of stored samples & 6 cases of farm ones the contamination exceeded Maximum Residue Limit ,i.e. 81/42% , 38.88% . Recovery test at two levels (0.1 and 1 ppm) with 3 replicate was done and the result average was 94.8% .So this method provides reliable and accurate results and it shows that they will be a serious threat to health if they are not properly controlled.

Keywords:*Diazinon, Pesticide Residue, Solid Phase Extraction.*

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PS7TH405

Others

P7

Epidemiological aspects of human myiasis in Iran

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Myiasis is a common disease in Iran. In this study we evaluate knowledge, community perceptions and practice about myiasis and also find medical importance of human myiasis in Fars province. A descriptive cross sectional survey using semi-structured questionnaire was used in the study area. A total of 302 cattle keepers, shepherd and butchers questioned randomly in the area of study. Data were analyzed by SPSS 16 software. Near to 30% of the surveyed population were illiterate. Majority of them(88.3%) have been stricken at least once myiasis during their job experiences. The frequency of the disease in the patients was 1 to more than 30 times. Close to 71.5% of them had been infected out of the barns. Pharyngeal myiasis can be expected in at least 87% of patients. The causing agent for the patients was sheep botfly, Oesteru ovis (Diptera: Oesteridae). Continuously painful throat irritation, itching, watery eye and runny nose are the common symptoms of the disease. Duration of remission of the disease was more than 5 days. Despite of more variation in academic educational levels of the surveyed community, their health educational level seems to be more similar. Age and job experiences had effect neither on their knowledge nor on their practice. Their academic educational level also had no significant effect on their knowledge but it had significant effect on their practice. It can be concluded that a comprehensive and continued educational program is needed to aware the people about myiasis, its prevention and control.

Keywords:*Human Myiasis, Nasopharyngeal myiasis, Oesterus ovis, Diptera, Fars province, Iran*

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PS7TH406

Others

P7

Chemical analysis of spinach resistance to aphids and leaf miners

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Host plant resistance is an essential factor of integrated pest management (IPM) while understanding the biochemical mechanisms could speed up the resistance breeding. To study the biochemical mechanisms of spinach plant resistance to its major insect pests, leafminer (Liriomyza langei) and aphids, sixteen Spinach genotypes with various degree of resistance were planted in growth chambers and a field with four replications. Sugars, protein, chlorophylls, carotenoids, and phenolics were extracted from plant leaves, and damage by leafminers and numbers of aphids in different species were recorded. Leafminer stings per cm2 leaf area of young plants were highly correlated with sting/cm2 of mature plants. This suggests that leafminer-resistant varieties for mature spinach plants may also be deployed for baby leaf production. Sucrose concentration of young plants was correlated with leafminer stings per cm2 leaf ($r = 0.729$, $p = 0.01$) and mines per plant ($r = 0.789$, $p = 0.01$), suggesting that low sucrose content could be used as a marker for early screening or selection for leafminer resistance. Leafminer stings/cm2 were negatively associated with contents of flavonoids ($r = -0.558$, $p = 0.05$) and chlorogenic acid ($r = -0.5978$, $p = 0.05$), while mines per plant were negatively associated with concentrations of flavonoid ($r = -0.701$, $p = 0.01$), chlorogenic acid ($r = -0.592$, $p = 0.05$) and rutin ($r = -0.601$, $p = 0.05$), implying a role of phenolics in the leafminer resistance.

Keywords:*Host plant resistant, leafminer (Liriomyza langei) and aphids, sugars,phenolics, chlorophy*

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PS7TH407

Others

P7

Biology of *Phalantha phalantha* duraury (Lepidoptera : Nymphalidae) an injurious pest of well known medicinal plant *Withania somnifera* Dunal

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Studies on the biology of Phalantha phalantha Drary (Lepidoptera: Nymphalidae) has been recorded on the host plant of Withania somnifera Dunal (Ashvangandha). The gross morphology and duration of various developmental stages has been recorded. The larval and oviposition behaviour has been studied in detail. Nectar food plants of the adult and ontogenetic variability in larval stage has been recorded. The adult butterfly is orange colored with black markings on the wings. Length of female butterfly is 18.67 ? 0.24 mm with a wing span of 56.33 ? 0.16mm. The length of male butterfly is 13.8 ?0.34 long and the wing expanse is 45.20 ? 0.43 mm.

Keywords:*Phalantha phalantha, Withania somnifera, Nymphalidae, Medicanal plant, Instars, Pupa, Butterfly, P*

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PS7TH408

Others

P7

Survey of relationship between birds damage and morfological characteristics of sunflower

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Survey of the caused damage by the birds on the sunflower fields in different planting dates in west Azarbaijan Esmaeil Alizadeh¹(Email:ISM478@Yahoo.com) and nouraddın shayesteh 1 - Agricultural and Natural Resources Research Center of West Azarbaijan. Urmia--P.O.Box : 365. 2-islamic azad university,mahabad branch-mahabad-iran Birds are among the most important pests in the fields under sunflower cultivation that lead to huge annual losses of the crop. Out of the deleterious birds of sunflower fields, house sparrow (Passer domesticus) causes the most abundant damage in the regional fields of sunflower. The present investigation was carried out to evaluate house sparrow damage on sunflower cultures in the Agricultural and Natural Resource Research Station of Khoy since 2006 in a biannual research project. The main plots included four seeding dates with 15 day intervals and the accessory plots were three sunflower cultivars Alstar, Euroflor, and KSC43/128. The cultivars were seeded in the Khoy station and the rates of bird damages were calculated at the end of seed maturity period. Based on variance analyses of data, seeding dates and cultivars had highly significant impacts on sunflower yield and the damage rate. The highest protected yields were resulted from the first and the second seeding dates exceeding 2500 kg/ha. However, the most damage rates were also calculated with the first and the second seeding dates, respectively rated as 96% and 93%. The damage rate in the second year was higher than that in the first year. Among the tested cultivars, the hybrid Alstar was of the highest

Keywords:*Sunflower, Damage and Birds*

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Others

P7

Quantitative and qualitative wheat loss assessment at different storage structures

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A laboratory experiment was conducted to evaluate the qualitative and quantitative wheat loss assessment in different storage structures. Cereal grains are the major source of food for humans and most domesticated animals. Wheat storage losses were assessed by sampling wheat from five storage structures (Tandlianwala, Mamookanjan, Buchiana, Jaranwala and Sumundri). Quantitative losses were assessed by counting and separating insect pest infected damaged, black headed, shriveled, broken grains and foreign matter in wheat at average or percentage basis which showed that stored wheat has more damaged and insect infested grains then fresh wheat sample. Wheat samples were chemically analyzed to calculate the loss in quality by comparing their Ash percentage, Moisture percentage, Percent protein contents and Percent fat contents with the fresh wheat samples. Moisture contents were having the mean 8.489 % in fresh wheat stock and 10.452 % in stored wheat. The protein contents were having the mean 13.763 % in fresh wheat stock and 11.333 % in stored wheat. The crude fat contents were having the mean 5.812 % in fresh wheat stock and 4.925 % in stored wheat. The ash contents were having the mean 1.327 % in fresh wheat stock and 1.215 % in stored wheat.

Keywords:*loss assessment*

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Others

P7

Investigation of genetic diversity of *Hygrobates fluviatilis* (Hydrachnidia: Hygrobatidae) populations from different habitats in Iran

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The genetic diversity of water mite Hygrobates fluviatilis (Acari: Hydrachnidia) evaluated based on eight microsatellite loci (HfMS01, HfMS02, HfMS08, HfMS09, HfMS014, HfMS029, HfMS033 and HfMS040; Asadi et al., 2009). Eight populations collected from two different types of habitats (springs and streams) were evaluated. Data were analyzed based on UPGMA method using Popgene software. The number of alleles detected ranging from 2 to 21 (average, 9.23 per locus). The observed heterozygosity ranged from 0.063 to 0.813. Of 13 primers, four populations from spring and four from stream were tested and 292 alleles were totally detected. The total amount of gene diversity ranged from 0.722 (locus Hf09) to 0.945 (locus Hf29) and 0.872 (average per locus). Eight populations were detected in two separate groups. The genetic distance between habitats was high, which means that the genetic differences were caused by habitat differences. The results also indicated that polymorphic microsatellites provided a clear differentiation between spring and stream populations which was not found in our previous study based on mitochondrial cytochrome c oxidase subunit I (COI) gene (Asadi et al., 2012). Asadi M, Higaki T, Hinomoto N, Saboori A, Naghavi MR (2009) Isolation and characterization of polymorphic microsatellite loci from the water mite Hygrobates fluviatilis (Acari: Hydrachnidia: Hygrobatidae). Molecular Ecology Resources 9:793-795. Asadi M, Hinomoto N, Saboori A, Javan-Nikkhah M (2012) Genetic diversity in mitochondrial cytochrome c oxidase subunit I sequences of the water mite Hygrobates fluviatilis (Acari: Hydrachnidia: Hygrobatidae). International Journal of Acarology38:96-100.

Keywords:*water mite, microsatellite loci, genetic diversity, population, Iran*

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Others

P7

A preliminary documentation of consumable Acridid (Orthoptera) species worldwide

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Insects are seldom been seriously considered as possible nutrient source for animal husbandry or for human consumption, although scattered literature supports that being of its high protein content some insects are used as feed or feed ingredient or for human consumption since long. Among insects, grasshoppers and locusts (Orthoptera) have some advantageous properties in favour of entomophagism or feed component. The present investigation performed data-mining, using DORSA (http://www.dorsa.de) and OSF (http://orthoptera.speciesfile.org) and isolated 55 Acridid species (Orthoptera) consumed by people from 29 countries worldwide. We identified the species mainly from two museum sources from Bonn and Berlin, Germany. Mean length, pronotum-width, head-width of both sexes of 55 species with their corresponding volumes were measured. Among 55 species the longest species lengths (mm±SD) were 47.29±1.24 (among males) and 67.7±3.28 (among females) where the shortest species lengths were 14.17±1.11 (among males) and 16.17±0.7 (among females). The minimum and maximum head-widths (mm±SD) were recorded as 1.77±0.18 and 6.2±0.07 (males) and 2.57±0.35 and 7.82±0.37 (females). Out of 55 species, nutritional values of some fresh available species/genus were estimated in laboratory and that estimations for the rest unavailable species/genus were tabulated using available published records. Generally Orthoptera do have 22.8% (minimum) to 65.39% (maximum) crude protein with an average of 44.1% (dry matter) and those findings very much corresponds with our results (60.2% to 68.1%). Once the full compilation of data, principally culture procedure, breeding potential and nutritional values of recognized species are available, viable species of Acridids to be cultured economically can be selected.

Keywords:*global consumable acridid, morphometry, nutritional value*

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Others

P7

Species richness & abundance of Arctiinae (Erebidae: Noctuoidea) in Western Ghats of India

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The Western Ghats of India are acclaimed as one of the biodiversity hot spots of the Globe. Originating from the mouth of river Tapti (Gujarat) & running along the West coast of India, they cover an area of 160,000 sq. km & stretches for 1600 km to Kanyakumari in Tamil Nadu. Being one of the richest areas of biological wealth, Western Ghats exhibit a vast range of biotic provinces coupled with high degree of endemism. Insects, which constitute 66% of the total animal kingdom, play a vital in the development & maintenance of such biotic provinces with which the future of humans is inextricably linked. Present study deals with the species richness and abundance of such an economically important group of insects i.e. arctiinae (Erebidae: Noctuoidea: Lepidoptera) from Western Ghats of India. More than 40 localities of Western Ghats were selected as sample sites, to which, repeated collection visits yielded a total number of 1650 representatives of 101 species under 46 genera of arctiinae. Two genera and eight species were reported for the first time from India whereas, the extended distributional limits of other eleven species were also reported. It was observed that some of the localities were proved to be excellent for arctiin diversity and others were restricted to few species or specimens. This research work is first of its kind on arctiin moths from Western Ghats of India and definitely, an addition to the existing knowledge about this group. The details are discussed.

Keywords:*Arctiinae, species richness & abundance, Western Ghats, India*

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Others

P7

Antimicrobial activity and composition of *Artemisia judaica* L. essential oil from Egypt

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Artemisia judaica is a widely distributed plant in the Middle East (Sinai Peninsula, Jordan and Saudi Arabia). The antimicrobial activity of essential oil derived via hydro-distillation method from aerial parts of A. judaica L., were evaluated for antimicrobial activities. Two Gram-positive bacteria (Bacillus subtilis and Streptomyces spp.); one Gram-negative bacterium Escherichia coli O157; five fungi strains (Fusarium oxysporum, Rhizopus stolonifer, Rhizoctonia solani, Macrophomina phaseolina and Aspergillus niger) and three yeasts (Candida albicans, Candida tropicalis and Saccharomyces cerevisiae) were used for evaluation of antimicrobial activities of A. judaica essential oil. The agar gel diffusion method was used to assay for the antimicrobial properties on the test isolate. The activity was more pronounced against Gram-positive and candidal than against fungal organisms except R. stolonifer. The chemical composition of the essential oil was analyzed by GC-MS and the resulting oil contained piperitone (32.4%), camphor (20.6%) and E-ethyl cinnnamate (8.2%) as major components, which may provide the antimicrobial properties of the essential oil against tested organisms.

Keywords:*Artemisia judaica, Essential oil, Antimicrobial activity, GC, MS analysis, Chemical composition*

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Others

P7

Pollen analysis in honeys collected from Karnataka region of Nilgiri Biosphere, South India

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The present work refers to the pollen analysis of 15 honey samples collected from three locations from Karnataka region of Nilgiri Biosphere, South India. The honey samples were collected from the domestic bee hives of Apis cerena and wild bee hives of Apis dorsata from local honey hunters of forest most represented families. Ther was a dominance of tree species which are the most preferred and highest contribution for nectar and pollen source for honeybees in the study area. This study discusses regions during March 2010 to October 2011. Pollen analysis was performed using acetolysis method . the samples were subjected to both qualitative and quantitative analysis. Out of 15 samples analyzed one sample was recorded as unifloral, which contains Coffea sp., as predominant pollen and remaining 14 honey samples were recorded as multifloral. Fabaceae, Asteraceae and Myrtaceae were the honey plant resources and potentialities for commercial beekeeping in Nilgiri Biosphere.

Keywords:*Beekeeping, Honey plants, Melissopalynology, Pollen analysis.*

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Others

P7

Phisicochemical characteristics and pollen analysis of honeys from western ghats of Karnataka, South India

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The present study intended to determine a quantitative and qualitative analysis of 30 honey samples collected from honeybee colonies in different locations of Western Ghats of Karnataka, Out of which, 9 samples were found to be unifloral and 21 were multifloral. In unifloral honey samples, Sapindus laurifolia, Hevea sp., Areca catechu, Acacia sp., Mangifera indica, Terminalia bellerica, Syzygium sp., Pongamia pinnata, and Eucalyptus sp., were predominant pollen types. These samples were also analysed for several physicochemical parameters and recorded. The honey samples had moisture ranged from 7.4% to 32.5%, ash varied from 0.16% to 0.85%, pH in the range of 3.2 to 4.5, free acidity from 7.0meq kg to 39meq kg, reducing sugar from 60.2% to76.5%, total sugar from 64.2% to 81.6%, proteins from 0.22mg/100ml to 0.95mg/100ml, alkaloids from 2.0% to 19% and phenols from 20mg/100g to 96mg/100g. This type of physicochemical and pollen analysis of honey samples favors the possibility of utilizing the rich flora of Western Ghats for good quality honey.

Keywords:*Alkaloids, Free acidity, Phenol, Physicochemical, Pollen analysis, Proteins, Honeybees*

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Others

P7

Determination of major constituents of honeybee venom from Korea

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The aim of the study was to the determination quantitatively of the major constituents of honeybee (Apis mellifera L.) venom from Korea by liquid chromatography. Samples of honeybee venom were purchased from Chungjin, Ibee and Sigma over two consecutive beekeeping season(2007-2008). The sephadex TM75 and source 15RPC ST column was applied. Several honeybee venom constituents were separated and the content of three of them (melittin, apamine and phospholipase A2) were determined. The chemical composition of honeybee venom from Korea were evaluated. Melittin content varied from 48.5 to 67.2 and averaged 50.7%. Apamin content camewithin a range of 2.0 to 3.2, and averaged 2.8%, and phospholipase A2 content was between 10.8 to 14.5, averaging 12.8%. There is no statistically significant differences for the content of the main component compared standard honeybee venom to domestic honeybee venom. The quantitative composition of melittin, apamine and phospholipase A2 may provide a basis for the development of the domestic standard for honeybee venom.

Keywords:*bee venom, Liquid chromatography, melittin, apamin, phospholipase A2*

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PS7TH417

Others

P7

Royal jelly reduces melanin synthesis through down regulation of tyrosinase expression

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For cosmetic reasons, the demand for effective and safe skin-whitening agents is high. Since the key enzyme of the melanin synthetic pathway is tyrosinase, many depigmentating agents in the treatment of hyperpigmentation act as tyrosinase inhibitors. In this study, we have investigated the hypo-pigmentary mechanism of royal jelly in mouse melanocyte cell line, B16F1. Treatment of B16F1 cells with royal jelly markedly inhibited malanin biosynthesis in a dose-dependent manner. Decreased melanin contents occurred through the decrease of tyrosinase activity. The mRNA levels of tyrosinase were also reduced by royal jelly. These results suggest that royal jelly reduces melanin synthesis by down regulation of tyrosinase mRNA transcription and serve as a new candidate in the design of new skin-whitening or therapeutic agents.

Keywords:*royal jelly, Melanogenesis, Pigmentation*

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PS7TH418

Others

P7

Silk fibroin for guided bone regeneration

HaeYong Kweon
National Academy of Agricultural Science, Republic of Korea

Silk fibroin (SF) is a natural protein polymer spun by silkworm Bombyx mori and used as a typical textile fiber and a surgical suture. Recently, cytocompatibility and tissue-compatibility of SF has been investigated and elucidated that there is no severe side effect to cell and tissue. The author examined the feasibility of SF as eardrum membrane and it is good for regeneration of eardrum with high efficiency. To expand the usability of SF, the author examined SF for guided bone regeneration. SF membrane with bioactive materials was pre-pared and examined the morphology, structural characteristics, and thermal properties. And then it was applied to white rabbit to evaluate bone formation. Histomorphology and removal torque were analyzed.

Keywords:*sericulture, silk, fibroin, tissue engineering*

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Preparation and characterization of regenerated *Antheraea pernyi* silk fibroin

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Antheraea pernyi silk fiber was dissolved in calcium nitrate solution with various dissolving conditions. The solubility was significantly dependent on the concentration of calcium nitrate, dissolving temperature and time. The aqueous solution of Antheraea pernyi silk fibroin was composed of a mixture of polypeptides with several molecular weights of above 14 kDa. FTIR and XRD showed that regenerated Antheraea pernyi silk fibroin was composed of a α-helix as well as random coil conformation while silk fiber was a traditional β-sheet structure. The endo-exo transition in temperature ranges of 228~232℃ also supports these conformations of regenerated silk fibroin film. TGA and DTG curves showed that thermal decomposition of regenerated Antheraea pernyi silk fibroin proceeded by three steps. The mechanical damping peaks (tan δ) were appeared at about 227℃ with minor shoulder maximum at about 240℃, which were somewhat lower than those of tussah silk fiber.

Keywords:*sericulture, silk, tussah*

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PS7TH420

Others

P7

Thick flexible silk fibroin biological membrane

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Silk is a natural polymer with biocompatibility and low inflammatory reaction. Recently, Silk fibroin has been studied in the biomedical application. In this study, we made thick silk biological membranes containing different concentrations of salt and examined characteristics of the membranes. Until now there was a limit to produce a thick silk membrane which has a thickness of more than 100 micrometer. But we overcome the existing difficulties and were successful in producing a thick silk biological membrane. We had produced a silk membrane with thickness of more than 500 micrometer. Salt addition increased the transparency and maximum stretching length of silk membrane. The membranes did not show cytotoxicity. These silk membranes are expected to be use as medical application for variety of purposes.

Keywords:*sericulture, silk, membrane*

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PS7TH421

Others

P7

Pollinating activity of 3 species of insects, *A. mellifera, O. cornifrons*, B. *terrestris*, in Japanese apricot field

In Gyun Park[1], Mi-Ae Kim[2], Hyung Joo Yoon[3], Kyeong Yong Lee[4]

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To increase the fruit quality of the Japanese apricot, 3 species of insects, Apis mellifera, Bombus terrestris and Osmia cornifrons were used for pollination in Japanese apricot field in Jinju and Sancheong province. And also economical effect for crops of Japanese apricot fruit pollinated with same insect was analyzed. This study was also conducted using 3 kinds of methods for pollination effect, natural condition, net-house and control method. Because blooming period of Japanese apricot was different from the other fruit tree(Blooming time of Japanese apricot shows always in the middle of the March), foraging behavior of insects used in this examination showed different tendency each other. Especially O. cornifrons was not adapted to wind and lower temperature compared with A. mellifera and B. terrestris in this field condition. Rate of fruit set pollinated with A. mellifera and B. terrestris was showed 14.9% and 13% respectively. Total rate of fruit set of Japanese apricot showed lower tendency compared with other kinds of fruit tree with under 30%. Quality of fruit pollinated with A. mellifera and B. terrestris showed higher than O. cornifrons in natural field condition in fruit weight.

Keywords:*Japanese apricot, Pollination, Apis mellifera, Bombus terrestris, Osmia cornifrons*

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The influence of soil exposure methods on toxicities of insect growth regulators (IGRs) for *Paranura rosea* (Collembola)

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Only information about mortality and juvenile production is obtained in existing Collembolan reproduction test. In this study, toxic effects of the three insect growth regulators (teflubenzuron, fenoxycarb, tebufenozide) were evaluated in the OECD artificial soil for determining potential and reliability of new exposure method. Several tests (1-2) were conducted. 1) The reproduction test using Paranura rosea was conducted in two different exposure conditions. One was existing method (ISO standard method) and other which was exposed in the compacted soil is new soil exposure method (surface contact only method). Several endpoints were examined after 28-day exposure. 2) Also some endpoints were compared with the result of solid leachate phase test using Microtox® for investigating toxicity change by time. In ISO standard method, median lethal concentration (LC50) for adult and median effective concentration (EC50) for juvenile was estimated, and in surface contact only method, LC50 for adult, EC50=egg for egg production and EC50=juvenile for hatched juvenile was estimated. Teflubenzuron which is the most toxic chemical showed high toxicity at all the end points. and fenoxycarb showed high ovicidal effect. Especially, for fenoxycarb, toxic effect was reduced as time passed. Tebufenozide showed relatively low toxicity. However, the toxicity of all the IGRs in ISO standard method was more toxic than that of the surface contact only method. Thus, to compare the result in ISO standard method with that in surface contact only method is good enough to trace the specific toxic effects of the test chemicals and provide additory endpoints.

Keywords:*Paranura rosea, Collembola reproduction test, Insect growth regulator, New exposure method*

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Complete comparative genomic analysis of *Bombyx mori* nucleopolyhedrovirus-K1, K4 and K5 strains isolated in Korea

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The genome of three genetically distinct isolates of Bombyx mori nucleopolyhedroviruses (BmNPVs), BmNPV-K1, K4 and K5 strains isolated in Korea was completely sequenced and comparative analyzed. BmNPV-K1 consisted of 126,747 bp and 131 open reading frames (ORFs) of 150 nucleotides or longer with minimal overlap have been identified. In contrast, BmNPV-K4 and K5 consisted of 128,618 bp and 134 open reading frames (ORFs), 127,554 bp and 133 open reading frames (ORFs), respectively. Although gene arrangement is virtually identical, the genome of BmNPV-K4 was 1,871 bp and 1,064 bp longer than that of BmNPV-K1 and K5, respectively. The most interesting difference between these viruses was the presence or absence of baculovirus repeated ORFs (bro) genes. To investigate the relationship between BmNPV-K1, K4 and K5, phylogenetic analysis with each member of the paired ORFs was performed. The sequence data suggest that BmNPV-K1, K4 and K5 are closely related but have diverged and evolved into three separate strains. This was study to identify highly related but separately evolving viruses in the same insect host and geographic location. We are currently comparing the differences of these BmNPV genomes to elucidate function of novel genes.

Keywords:*Bombyx mori, nucleoplyhedrovirus, bro gene*

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Production of porcine circovirus type 2 capsid protein by the fusion expression with baculovirus partial polyhedrin in insect cells

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Porcine circovirus type 2 (PCV2) is a non-enveloped circular single-stranded DNA virus associated with postweaning multisystemic wasting syndrome, which is considered to be an important infectious swine viral disease. PCV2 capsid protein encoded by ORF2 is a major structural protein and expected as the high immunogenicity protein. For the efficient production of capsid protein, the recombinant baculoviruses were generated to express ORF2 of various fusion forms with a partial polyhedrin of Autographa californica nucleopolyhedrovirus. Recombinant capsid protein was produced successfully with fusion form of partial polyhedrin and the yield was high like as shown visible clearly on SDS-PAGE. Production and status of recombinant fusion proteins in insect cells were confirmed by SDS-PAGE and Western blot analysis using His-tag antibody, anti-ORF2 monoclonal antibody and anti-PCV2 serum. Optimal multiplicity of infection (MOI) and infection time for the production of recombinant capsid protein were determined as 5 MOI and 4 days, respectively. The results suggest that the fusion expression with polyhedrin is able to increase the production of recombinant PCV2 capsid protein in insect cells.

Keywords:*Baculovirus, Capsid protein, ORF2, Polyhedrin, Porcine circovirus type 2*

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Antimicrobial effects on *Propionibacterium acnes* of extracts from Korean propolis

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Propolis, or 'bee glue', is a complex resinous mixture of plant-derived products gathered, modified and used by bees as a general purpose sealer, draught excluder and antibiotic in their hives. The presence of propolis within the hive may also provide an environment not suitable for the growth of bacteria and other microorganisms. Propolis composition is directly related to that of bud exudates collected by bees from various trees. The composition of the propolis depends upon the vegetation of the area from where it was collected on the bee species. Propolis is an excellent material, as known as antimicrobial activity, propolis samples were collected in south korea, propolis extracted with ethanol(EEP), EEP was fractionated by prepHPLC, and antimicrobial activity against acne bacteria (Propionibacterium acnes) investigated. Antimicrobial activity was detected in the 2-3-5-4 fraction.

Keywords:*propolis, antimicrobial effect, P. acne*

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Development of 「Ecology experience kit displaying photo-tactic insects」 to activate the insect industry

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It was carried out to develop the ecology experience kit displaying the photo-tactic insects to activate the insect industry. The kit was developed to have the characteristics of being moved with wheels in plain place and carrying by truck, and being used semi-permanently. To display the insects and plants, this kit was equipped with the electric lighting, natural ventilation and drain system. The 8 species of insects and 7 species of plants were selected as adaptable insects and plants for the kit. In the 8 species of insects, Pieris melete and Eurema hecabe were very suitable in the kit because of their small size and vivid flying activity. In particular, Baculum elongatum has several peculiarities like parthenogenesis, unique external features and dropping oviposition, it seemed to have worth as a industrial pet insect. The kit was expected to remind adults of nostalgia, to offer students the nature sense, and to increase the income of industrial insect farmers.

Keywords:*Ecology experience kit, insect, Pieris melete, Eurema hecabe, Baculum elongatum*

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Trapping Time Effect on a Korean Strain of the Entomopathogenic Nematode, *Steinernema carpocapsae*

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A Korean isolate of the entomopathogenic nematode (EPN) Steinernema carpocapsae KCTC 0981BP strain (ScK) is effective for control of many Korean agricultural and forestry pests. In vitro culture is available for large scale of mass production of commercial EPN, but it is a costly and complicated process, whereas in vivo culture using great wax moth Galleria mellonella larvae is simpler for small scale production. However, trapping time during in vivo culture may influence harvesting and survival of EPN. We investigated effects of this factor on harvest, survival, and pathogenicity of ScK. Elapsed time to first and maximum emergence of progeny (TFE and TME, respectively) from G. mellonella larvae was highly influenced by trapping time on the White trap. Emergence time was shorter with trapping on the White trap. The TFE was shorter in the 7th to 9th day trapping. TME was shorter in late trapping. Trapping time did not influence duration of emergence. Mean number of progeny was not significantly affected by trapping time except on the 9th day trapping.

Keywords:*Steinernema carpocapsae, Steinernemidae, white trap, in vivo production*

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Others

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Cloning and characterization of *Pieris rapae* granulovirus chitinase gene

Seunghan Oh[1], Dong Hyun Kim[2], Bharat BhusanPatnaik[3], Yong Hun Jo[4], Mi Young Noh[5], HyoJeong Lee[6], Kwang Ho Lee[7], Kwang Ho Yoon[8], Wan-Jong Kim[9], Ju Young Noh[10], Hun Cheon Jeong[11], Yong Seok Lee[12], Chuan-Xi Zhang[13], In Seok Bang[14], Yeon soo Han[15]

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The chitinase gene of baculovirus is expressed in late phase of virus replication in insects and possesses high exo- and endo-chitinase activity, which can hydrolyze inherent chitin in insect body, thus promoting terminal host liquefaction. Nucleopolyhedrovirus (NPVs) viral chitinases (vChitA) have been well analyzed, but information is limited as regards to vChitA from granuloviruses (GVs). Whole-genome sequencing of the Korean isolate of Pieris rapae GV proposed a putative chitinase gene corresponding to ORF10. The chitinase gene had a size of 1761 nt, potentially encoding a protein of approximately 65.6 kDa. The deduced amino acid corresponded to a size of 586 amino acids with a putative 19 amino acid putative signal peptide at the N-terminus. Temporal expression analysis of PrGV chitinase transcript in infected Pieris rapae larvae by both semi-quantitative and real-time PCR revealed significant 75 fold increase after four days of infection followed by decline at the latter stages of infection. In addition, time course induction pattern observed by SDS-PAGE followed by subsequent Western blotting with anti-PrGV chitinase antibody revealed the dissociation of the signal peptide from the intact chitinase. N-terminal sequence analysis further confirmed the exact nature of the mature chitinase depicting amino acid residues just after the signal peptide. Furthermore, immunofluorescence studies indicated strong chitinase-specific signals in fat body cells and epithelial cells associated with integument at four days post infection with its assumed significance as secreted protein, possibly due to the lack of ER retention motif (KDEL) in its sequence. Finally, immunogold labeling and electron microscopy studies reflected

Keywords:*Pieris rapae granulovirus, viral chitinase, cloning, confocal microscopy*

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Others

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Molecular and Immunohistochemical characterization of granulin gene encoded in *Pieris rapae* granulovirus genome

Seunghan Oh[1], Dong Hyun Kim[2], Bharat BhusanPatnaik[3], Yong Hun Jo[4], Mi Young Noh[5], HyoJeong Lee[6], Kwang Ho Lee[7], Kwang Ho Yoon[8], Wan-Jong Kim[9], Ju Young Noh[10], Hun Cheon Jeong[11], Yong Seok Lee[12], Chuan-Xi Zhang[13], In Seok Bang[14], Yeon soo Han[15]

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Pieris rapae granulovirus is highly pathogenic to the cabbage butterfly (Pieris rapae), an important pest of cultivated cabbages and mustard crops. It therefore holds significant promise towards exploitation as a potent bio-control agent in the field controlling the pest population. Whole-genome elucidation of the Korean isolate of the granulovirus, reported the presence of a functional granulin gene corresponding to ORF 1 in its genome. Comprehensive studies towards functional characterization of the gene, established that itis composed of 744 nucleotides and encodes a peptide of 247 amino acid residues. It possessed significant homology with AoGV and ClanGV with 87% identity at amino acid level. Multiple alignment data suggests that the C-terminus region of the gene had three different conserved regions. Time-course studies conducted in PrGV infected Pieris rapae larvae revealed a significant upsurge of the transcript (134 fold) at four days post infection followed by a significant decline at the most advanced stages of infection. Anti-PrGV granulin antibody was produced and western blot conducted with the infected larvae further confirmed the induction pattern with a protein of 30K. Immunofluorescent staining showed a granulin-specific signal in fat body and integument of the infected larvae.Granulin-specific signals were noticed two days post infection with the eventual systemic spread of infection to the associated tracheal matrix witnessed at four days post infection. Immunogold labeling and electron microscopic studies further proved the cytopathological effects as the presence of numerous membrane-bound vesicles with nucleocapsids and abruption of intercellular junctions in fat body and hypertrophied cells in the

Keywords:*Pierisrapae, Pieris rapae granulovirus, granulin, gene expression, immunofluorescent microscopy*

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Cloning and genomic structure of superoxide dismutade-1(sod1) gene from the entomopathogenic fungus, *Cordyceps pruinosa*

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We have cloned Superoxide Dismutase-1 gene by PCR from the entomopathogenic fungus, Cordyceps pruinosa, and sequenced and analyzed the complete nucleotide sequence and genomic structure of the gene.The SOD1 gene of Cordyceps pruinosa spans 947bp and consisted of 4 exons and 3 introns coding for 154 amino acid residues. The three different introns in length separate exons of 13, 331, 97 and 20bp. The deduced amino acid sequence of C. pruinosa SOD1 showed 95% identity to Beauveria bassiana and Cordyceps militaris. Phylogenetic analysis showed that C. pruinosa SOD1 is placed in the Ascomycota group of fungal clade.

Keywords:*Cloning, Genomic structure, SOD, Entomopathogenic fungus*

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Effects of aqueous extracts of basil, *Ocimum basilicum* L., Sodom's apple, *Calotropis procera* Ait and Coriander *Coriandrum sativum* L. Flea beetle *Podagrica* spp. on okra crop

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Rehab E. H. Fadwal, Faiza E. E. Salah and Mohammed H. Z. Elabdeen and Elamin M. E. Dept. of Crop Protection,Faculty of Agricultural sciences U. of Gezira, Sudan An experiment was conducted at the U. Of Gezira Experimental farm to evaluate the effects of 10% aqueous leaf and fruit extracts of Basil, (Ocimum basilicum L.), leaf extracts of Sodom's apple, (Calotropis procera Ait) and fruit extract of Coriander , (Coriandrum sativum L.) on Flea beetle Podagrica spp.on okra. The experimental design comprised 16 plots assigned to 4 treatments (replicated 4 times), arranged in a completely, randomized block design. The treatments consisted of spraying okra plants with Basil, Sodom's apple and Coriander 10% aqueous extract or distilled water (control). The efficacy of the extracts was assessed in terms of shot holes in okra leaves i.e., where Flea beetle larvae were alive and feeding. The results indicated that the three aqueous extracts significantly ($P<0.05$) reduced the infestation level by the Flea beetle on okra leaves. Coriander extract resulted in the lowest infestation level by the Flea beetle (mean number of shot holes =630), followed by Rehan (mean number of shot holes=711) and Usher (794) while the control treatment scored 1327 shot holes.

Keywords:*Aqueous extracts, okra, Flea beetle, Sudan*

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Effects of aqueous extracts of basil, *Ocimum basilicum* L., Sodom's apple, *Calotropis procera* Ait and Coriander *Coriandrum sativum* L. on leaf miner, Liriomyza spp., on okra crop

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Faiza E. E. Salah1, Elamin M. Elamin2, Elameen M. A. Eltoum1, Hayder Abdelgader2 and Dominique Bordat3 1 Dept. of Crop Protection, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan. P.O. Box 20 2 Agricultural Research Corporation, Wad Medani, Sudan. 3 CIRAD, Montpellier, France. Email: Faizaruba@yahoo Abstract: The Hymenopterous parasitoids, Hemiptarsemus varicornis (Girault) and Opius dissitus (Muesebeck) are associated with the leaf miner, Liriomyza spp., populations in Central Sudan. The effects of Liriomyza trifolii (Burgess) and Liriomyza sativae (Blanchard) reared on common bean, Phaseolus vulgaris, on the development and efficiency of their two parasitoids were studied at constant conditions of temperature, relative humidity and photoperiod. No significant differences were found between parasitism percentage of H. varicornis or O. dissitus on L. trifolii or L. sativae. However, significant differences were found with respect to adult (male and female) life span as well as the number of adult parasitoids emerged of H. varicornis and O. dissitus. The life span of H. varicornis was shorter than that of O. dissitus, but more adults of the later emerged from the pupae than those of H. varicornis which might have accounted for their almost equal parasitism percentages. Also, no significant differences were recorded with regard to the development of H. varicornis on L. sativae reared on four of its host plants. However, parasitism percentage was significantly higher on gourd (Cucurbita moschata) followed by zucchini (cucurbita pepo), haricot bean (Phaseolus vulgaris) and tomato (Lycopersicon esulentium), respectively.

Keywords:*Aqueous extracts, okra, leaf miner, Sudan*

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Molecular shift in insecticidal proteins: I. Evidence of beneficial trait deterioration in nematode-symbiotic bacterium complex

Cheng Bai

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Entomopathogenic nematodes are used for the biological control of soil pests and associated with mutualistic bacteria, e.g., Heterorhabditis bacteriophora with Photorhabdus luminescens. The nematode infective juveniles penetrate into the host bodies and release the bacteria. Bacteria grow and produce toxins that kill the host. Nematodes reproduce inside insect cadaver, feeding on bacteria. Biochemical study of the mechanism of trait deterioration in nematode-symbiotic bacterium complex at molecular level is crucial to explore novel and improve existing biological agents for better pest control. Serial in vivo culture with foundation population (FP) of mixed H. bacteriophora strain for 16 passages (each passage equals to two or three generations) results in a 30% loss in virulence, the beneficial trait for pest suppression. Protein analysis (1) on the SDS-PAGE reveals that the insecticidal toxin complex protein with a molecular mass of 283-kDa (toxin A, encoded by TcdA1 gene) is unstable at passage 16 and has been cleaved into 90-kDa insecticidal toxin protein subunits, and (2) on the 2-D gel demonstrates that both 330- and 220-kDa insecticidal toxin complex proteins are abnormally cleaved into 90-kDa protein subunits at passage 16, resulting in a apparently reduced amount of 110-kDa insecticidal toxin protein subunits when compared to the corresponding FP. The 90-kDa toxin protein is truncated form of the 110-kDa toxin protein. This result indicates a difference in the cleavage of the insecticidal toxin complex molecules between passage 16 and the FP.

Keywords:*Entomopathogenic nematodes, Photorhabdus luminescens,trait deterioration,insecticidal proteins*

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Characteristics of a BT strain with high insecticidal activity

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Up date, more than fifty thousands of Bacillus thuringiensis (BT) strains or isolates have been collected, but most of them have non insecticidal activity to pests. It is exciting very few BT strains in the world those have a high toxicity to certain of target pests. A new Bt strain (Bt 00-50-5) is isolated , which has a high activity against pests, e.g., 00-50-5 is about 250 times more toxic than HD-1 (The strain is used often for production) against the banded sunflower moth, Cochlis hopes; The LC50 (50% Lather concentration) is 0.5ppm and 0.2ppm for the mixture of spores and crystals from medium scale fermentation product, and for the purified crystal protein against fourth instar larvae respectively. The pure spores have no obvious to the pest with test concentrations, but the pure crystals show a high insecticidal activity. The bacterium also can control Pieris repae and Agrotis segatum pests. The major insecticidal proteins were characterized. The fermentation of the BT 00-50-5 could be completed within 23 hrs and the ratios of the released numbers of spores : crystals are about 1 : 2.5. BT 00-50-5 has a great potation for making commercial biological pesticides.

Keywords:*Bacillus thuringiensis, a new Bt strain (Bt 00-50-5), insecticidal activity,Characteristics*

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Chikungunya an alphaviral infection: Emerging threat in urban area of Bangalore, India

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Background: - Chikungunya virus is a rare form of genus alpha virus (Arbovirus), family-togaviridae which is spreaded by Aedes mosquitoes. Fever with erratic, relapsing and incapacitating arthralgia and arthropathy especially in the metacarpho- phalangeal, elbow, shoulder, knee, ankle, metatarsal joints are the prominent diagnostic features of chikungunya fever. Chikungunya fever outbreaks begin during rainy season when vector density becomes peak and temperature and humidity are favorable for survival of the vector. Method:-The study was conducted in J.J.R Nagar, Bangalore which consists of 200 houses and 1200 populations including migrant workers, temporary and permanent dwellers. Out of 200 houses, 162(81%) were surveyed by simple randomization according to Fisher table of random numbers. 140(98.60%) febrile patient's blood samples were collected in a sterile 5 ml disposable single use syringe. The blood samples were distributed into non-heparinized test tubes with proper labeling and transported to the PHI for Chikungunya IgM antibody test by ELISA method. Larvae samples were collected by following standard sampling procedure. Results:-Total 33(23.57%) cases were found positive for chikungunya IgM antibody. 224 larvae samples were collected for the study out of them 51samples were positive for larvae. Out of 51, 37 were positive for Aedes. 12 were positive for Anopheles and 2 were positive for Culex. Conclusion: - Chikungunya is emerging threat in urban area of Bangalore for last few years. The study revealed that elimination of breeding sites is the cost-effective approach to control of emerging chikungunya epidemic

Keywords:*Chikungunya, Aedes mosquito, Arthropathy, Immunoglobulin M*

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Epiblema strenuana: Challenges for its use as a biological control agent for the invasive plant *Parthenium hysterophorus* in Africa

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The annual weed Parthenium hysterophorus (Asteraceae: Heliantheae) (parthenium), has wide-ranging negative impacts on crop and animal production, biodiversity conservation, and human and animal health in Africa, Asia and Australia. Parthenium is an increasing problem in southern and eastern Africa, causing severe economic losses in some countries. In 2003, South Africa became only the third country globally, after Australia and India, to implement a biological control programme against this weed. Relying on the successful Australian programme, four insect agents and one pathogen were selected and imported into quarantine in South Africa, for evaluation of their suitability for release. Epiblema strenuana (Lepidoptera: Tortricidae) is one of the most widespread, damaging agents that has established on parthenium in Australia. However, this agent was rejected in India as it completed development on the oil-seed crop, Guizotia abyssinica (Asteraceae: Heliantheae), under laboratory conditions. As an introduction of E. strenuana into South Africa will undoubtedly extend beyond the country borders in time, as a matter of responsibility, initial host range testing in South Africa has focussed on determining the susceptibility of cultivars of G. abyssinica, an important native commercial crop in Ethiopia, to this agent. Under no-choice conditions E. strenuana completed development on all selected cultivars of G. abyssinica. However, in the presence of parthenium, no larval development occurred on G. abyssinica. The impact of these results and issues relating to further consideration of E. strenuana as a potential biological control agent for parthenium in Africa are discussed.

Keywords:*Parthenium hysterophorus, Epiblema strenuana, Biological control of weeds*

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The effects of Thai herbal essential oils on the oviposition-deterrent and ovicidal activities of *Aedes aegypti* (Linn), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say)

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King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand

The effect of oviposition-deterrent and ovicidal of seven essential oils were evaluated towards three mosquito vectors, Aedes aegypti, Anopheles dirus and Culex quinquefasciatus. The oviposition activity index (OAI) values of six essential oils namely Cananga odorata, Cymbopogon citratus, Cymbopogon nardus, Eucalyptus citriodora, Ocimum basilicum and Syzygium aromaticum indicated that there were more deterrent than the control whereas Citrus sinensis oil acted as oviposition attractant. At higher concentration (10%) of Ca. odorata (ylang ylang flowers) it showed a high percentage of effective repellency (ER) against oviposition at 99.4% to Ae. aegypti, 97.1% to An. dirus and 100% to Cx. quinquefasciatus, respectively. The results showed that the mean number of eggs were lower in treated than in untreated water. In addition, there was an inverse relationship between essential oil concentrations and ovicidal activity. As the concentration of essential oil increased from 1%, 5% and up to 10% conc., the hatching rate decreased. The essential oil of Ca. odorata at 10% conc. gave a minimum egg hatch rate of 10.4% (for Ae. aegypti), 0.8% (for An. dirus) and 1.1% (for Cx. quinquefasciatus) respectively. These results clearly revealed that the Thai essential oil of Ca. odorata served as a potential oviposition-deterrent and ovicidal against Ae. aegypti, An. dirus and Cx. quinquefasciatus.

Keywords:*essential oil, oviposition deterrent, Aedes aegypti, Anopheles dirus, Culex quinquefasciatus*

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Hyperparasitoids and biological control of insect pests: Differential vulnerability of primary parasitoids to obligate hyperparasitism can mitigate trophic cascades

Robert Sicelo Nofemela

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Obligate hyperparasitoids are considered an important ecological disturbance to biological control of insect pests, as they develop on primary parasitoids. While studies have traditionally considered direct interactions in simple food webs, an insect pest population can support several primary parasitoid species. Since primary parasitoids that vary in life-history traits can differ in vulnerability to hyperparasitism, it is desirable to establish if unaffected species can take advantage of reduced competition and increase their contribution to parasitism levels thereby mitigating effects of hyperparasitism to biological control. To investigate this question, I monitored populations of Plutella xylostella (Linnaeus), its primary parasitoids and hyperparasitoids on unsprayed cabbage plots at weekly intervals over seven consecutive years. Cotesia vestalis (Haliday) was a dominant primary parasitoid, as it accounted for 78.27% of total parasitism. However, it was a secondary host to three obligate hyperparasitoids: Mesochorus sp., Eurytoma sp. and Pteromalus sp. At low hyperparasitism levels, the higher efficiency of C. vestalis in utilizing younger host larvae limited host availability to other primary parasitoids. But, as hyperparasitism levels increased and its populations declined, populations of Oomyzus sokolowskii (Kurdjumov) and Diadromus collaris (Gravenhorst) increased significantly as they parasitized a greater proportion of available hosts. The ability of unaffected primary parasitoids to increase their contribution to total host parasitism resulted in insignificant influence of hyperparasitism on total primary parasitism and on infestation levels. This study shows that differential vulnerability of primary parasitoids to hyperparasitism can have a stabilising effect on biological control in one host - multiple primary parasitoid systems.

Keywords:*diamondback moth, primary parasitism, hyperparasitism, developmental traits, diversity, stability*

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Morphometric, molecular and biological studies of the *Thrips tabaci* Lind. (Thys: thripidae) populations collected from onion and tobacco in Iran

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The onion thrips has ability to form biotypes or subspecies in different geographical regions and on different host plants. In this study, 22 populations of this pest were collected from tobacco and onion in 17 provinces of Iran. 24 continuous variables were measured for each specimen on 631 slide-mounted females. The populations were compared using principal component and canonical discriminant analysis .In spite of some overlap,populations collected from tobacco were distinct from those collected from onion.Thrips individuals were projected on the first two principal components, which together accounted for 73.98% of overall variance. Eigenvalues of PC-I and PC-II were 15.81 and 1.20, respectively. The lengh ratio of 4th/7th antennal segments had the highest influence on PC-I. In canonical discriminant analysis, the first two functions contributed most to the separation between populations which together accounted for 98.38% of the total variation in the data. Also, 85.8% of original grouped cases were correctly classified. To assess population genetic structure of T.tabaci , genomic DNA was extracted using DNeasy Tissue and Blood Kit (Quiagene) .The mitochondrial cytochrom oxidase gene, subunit I (COI) was amplified and sequenced using C1-J-1751 and C1-N-2191 primers. The maximum parsimony, maximum likelihood and neighbor joining and bootstrap analyses were carried out using PAUP*. The most-parsimonious tree (length=168, RI=0.9129, CI=0.8631) and other trees obtained from other methods of analysis showed two distinct clades: 1)thrips collected from tobacco and 2) those collected from onion.The robustness of these clades was supported with the bootstrap values of 100% and 91%, respectively.

Keywords:*Thrips tabaci, onion, tobacco, Iran, Morphometric, molecular study*

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Study on population dynamics of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in Baluchestan

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Asian citrus psyllid, Diaphorina citri Kuwayama (Hem.:Psyllidae) is one of the most serious pest of citrus and is the vector of citrus greening disease. Population dynamism of D.citri was studied in Sarbaz, Rask and Polan, three regions in Baluchestan zone, Iran during 2007-2008. The terminal branches (25cm length) were evaluated and the relation among weather changes (temperature, humidity, rainfall), young flash density population dynamic was determined. Results showed that D.citri was presented throughout the year in studied area. Density of D. citri in regions Sarbaz and Rask was higher than Polan region. Temperature and young flash density showed positive correlation and humidity and rainfall showed negative correlation with population dynamism of D. citri.

Keywords:*Asian citrus psyllid,Diaphorina citri, population dynamism, temperature, relative humidity, rainfal*

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Efficacy of organic and synthetic insecticides on the control of cotton pests: The bollworm complex, *Helicoverpa armigera, Diparopsis castanea* and *Earias insulana* for small-scale farmers

Lawrence Nkosikhona Malinga

Agricultural Research Council, South Africa

A study was conducted during the 2006/2007 and 2007/2008 seasons to test different plant extracts for the control of Helicoverpa armigera, Earias insulana and Diparopis castanea larvae on cotton. Tobacco (Nicotiana tabacium), khaki weed (Tagetes minuta), thorn apple (Datura stramonium) and garlic (Allium sativum) were compared with Mospilan® (acetamiprid) and Decis® (Deltamethrin). During the two seasons, Decis® and tobacco treatments had a significant effect on the Helicoverpa armigera and Earias insulana larvae compared to the other treatments. The highest population reduction of Diparopis castanea larvae was observed in the plots treated with Decis®. The Decis® and tobacco treatments showed significant repellent and insecticidal activity against the bollworm complex (i.e. American, red and spiny bollworms). Plots that were treated with Mospilan® and Decis® had significantly higher cotton seed yields during the 2006/2007 and 2007/2008 seasons. Tobacco treatment gave significantly higher yield than the khaki weed, thorn apple and untreated control but did not differ significantly from the garlic treatment. The tobacco and garlic treatments gave lower yields than the Decis® and Mospilan® treatment but did not differ significantly from the Mospilan® treatment. On average Decis® and tobacco treatments exhibited significantly less bollworm larvae than the other treatments. Tobacco was the most promising biological pesticide against American and spiny bollworm larvae. These results suggested that plant extracts can be used as an alternative to reduce the bollworm larvae populations on cotton for small-scale farmers.

Keywords:*bollworm, small-scale farmers*

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Late instar western tent caterpillars thrive on tough mature leaves

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Spring-active, insect herbivores tend to emerge and begin feeding in synchrony with the budburst of their host plants. If leaf maturation is associated with physical and chemical changes in the leaves, which alter their nutritional suitability, asynchrony in egg hatch and budburst can be detrimental to larval success. This study evaluated the impact of leaf maturation on foliage quality of red alder trees for survival and development of western tent caterpillars (Malacosoma californicum pluviale). Mature leaves were tougher than young leaves. Larval responses to leaf maturation were instar-specific and while the survival and growth of early instars was not influenced by leaf age, fourth instar larvae survived better and developed faster on mature leaves than on young leaves. Overall developmental performance of the later instar larvae was higher on mature leaves. Our findings suggest that more rapid leaf maturation of sun-exposed trees is beneficial to western tent caterpillars and this could explain why they prefer to oviposit on the sunny sides of trees. Year to year variation in spring weather related to sun conditions could potentially modify populations of tent caterpillars by influencing food quality for later instars, but early instar larvae are little influenced by the phenology of leaf development.

Keywords:*phenology, insect fitness, leaf age, shading, climate change*

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Spectrum, stability and durability of monolocus and pyramided lines with green rice hopper [Grh] resistance genes

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Intenational Rice Research Institute, Philippines

Near Isogenic lines containing either one, two or three loci that code for resistance against the green rice leafhopper (Nephotettix cincticeps)[GRH] were evaluated for their resistance against other leafhoppers and planthoppers. Pyramided lines that contained grh2 and grh6 demonstrated general antixenotic resistance against four different hoppers including the green leafhopper [GLH], zigzag leafhopper (Recilia dorsalis)[ZLH], brown planthopper (Nilaparvata lugens)[BPH] and white-backed planthopper (Sogatella furcifera)[WBPH]; however, antibiotic resistance was effective only against GLH and GRL. This antibiotic resistance was not due to any induced resistance mechanism. Monolocus lines with Grh2, Grh4 or Grh6 had no apparent effect on GLH. Two pyramided lines (Grh2+Grh4, and Grh2+Grh4+Grh6) were highly resistant to GLH by reducing oviposition and survival. However, GLH reared successively on Grl2+Grl4 quickly adapted to the antibiotic resistance, but not the antixenotic resistance. Resistance in the pyramided line grh2+grh4 was stable as the plants aged, but likely caused a yield penalty expressed as lower rice grain weight compared with the recurrent parent lines.

Keywords:*Near-isogenic lines, planthoppers. leafhoppers, rice, genetics*

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Potential of *Paecilomyces lilacinus* for control of *Anastrepha ludens*

Ricardo Toledo Hernandez
El Colegio de la Frontera Sur, Mexico

Paecilomyces lilacinus is commonly fungi used to control phytopathogenic nematodes and insects, but its effect on fruit flies has not been investigated. We evaluated the pathogenicity of nine strains of P. lilacinus on adults of Anastrepha ludens, a pest that limits the production of citrus and mango in Mexico. To determine the pathogenicity, 8 days-old A. ludens adults were inoculated with a suspension of 1x108 conidia/ml under laboratory conditions. The virulence of two strains, CFFS-A53 and CFF-A60, was determined by estimating the LC50 and LT50 on flies of the same age. To evaluate the effect of infection on fecundity and fertility, flies were inoculated by spraying 15 pairs with a suspension of 1x1010 conidia/ml. It was shown that all strains were pathogenic but showed different degrees of virulence, from 28.8 to 52.4% mortality at 22 days after treatment and LT50 from 18 to 22 days. The LC50 for strains CFFS-A53 and CFFS-A60 was 3.10x102 and 1.48x107 conidia/ml, respectively. The infection of P. lilacinus reduced fecundity and fertility rates at 78.16% and 31.45% (CFFS-A53) and 35.62% and 18.52% (CFFS-A60), respectively. The long lethal times suggest that these strains could be used for horizontal transmission, using infected sterile flies as vectors. Likewise, sublethal effects on fecundity and fertility may contribute to reduce fruit flypopulation growing rates.

Keywords:*Sublethal effects, Biological control, fruit flies, bioassays, Entomopathogenic fungi*

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Forensic Entomology on the Gauteng Highveld, South Africa

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Forensic entomology utilises arthropods in legal investigations that involve death, neglect and abuse of humans or animals. After death there is a defined succession of arthropods that are attracted to the organism as it decomposes. The two main groups of insects with forensic importance are the Diptera (flies) and Coleoptera (beetles). Within the flies the Calliphora (blowfly) group is the most significant and the most abundant. The larval stages of the flies are very challenging to identify morphologically and where possible, are reared to adults for easier identification. Molecular techniques hold promise for faster and simpler identification of these insects. Species-specific sequence variation on ribosomal DNA (rDNA) is commonly used in molecular identification assays in insect species. The Internal Transcribed Spacer region 2 (ITS2) is a variable region within the rDNA and has been used to differentiate between closely related species. This study aimed to create an ITS2 based identification protocol for forensically important dipteran blowfly species present on the Gauteng Highveld, South Africa. Pig carcasses were used to attract insects which were sampled randomly. DNA extractions were performed on all the samples and the ITS2 regions amplified and sequenced. Based on these sequences, species-specific primers were designed.

Keywords:*Forensic entomology*

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Specific activity distribution of acetylcholinesterase and its gene ace2 expression in the bumblebee *Bombus hypocrita* (Hymenoptera: Apidae)

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Acetylcholinesterase (AChE) plays multiple roles in nerve conduction, and is the target site of organophosphorous and carbamate insecticides. In this study, the spatial and temporal activity of AChE and AChE gene ace2 expression in Bombus hypocrita were investigated. Results indicated that the AChE in the head showed the highest specific activity, 63.55% of the total AChE activities, while the thorax and abdomen 18.44% and 18.01%, respectively. The activity of AChE is in agreement with the relative quantitative expression of ace2 in the different tissues of B. hypocrita. The specific AChE activity of the subcellular organelles was, 42.77% in mitochondria, 23.36% in microsomes, 19.16% in cytoplasmic solution, and 14.43% in nuclei and cell debris. Both the activity of AChE and the relative quantitative expression of ace2 for adults were significant higher than that for larvae and pupae, but no significant difference was found among the adult workers at different ages. The lack of difference in the adults indicates that AchE activity was not significantly different between the foragers and nurses as well.

Keywords:*bumblebee, Bombus hypocrita, acetylcholinesterase, spatial and temporal distribution, ace2*

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Improved performance of Azadirachtin and Matrinee insecticides by plant oil for chemical control of the leafminer, *Liriomyza sativae* Blanchard (Dip: Agromyzidae) in cucumber

Ahmad Baghdadi[1], Fatemeh Saberfar[2], Aziz Shaekhi[3]

[1]PayamNoor University, Iran, [2]University of Tehran, Iran, Iran, [3]Ministry of Agriculture, Iran

The leafminer, Liriomyza sativae Blanchard, has become one of the most serious pests in greenhouses of vegetable crops throughout the world because of its short generation time, high reproductive rate, conspicuous damage and the ability to develop resistance to currently registered insecticides. In this study Azadirachtin 15% and kingbo 0.6 % (active ingredient: oxymatrine 0.2% and psoralen 0.4%) has been tested on L. sativae. Also a potent synergistic role of mineral oil with these insecticides was studied. These treatments were conducted on cucumber plants under laboratory conditions (25°c, 65 RH, L: D 16:8). The leaf-dip bioassay method was adopted for assaying azadirachtin and Kingbo as alone or mixed with mineral oil. The LC50 was estimated by POLO-PC, SAS software. The results indicated that the mixture of azadirchtin had a lower LC50 compared with insecticide alone (LC50 of azadirachtin and azadirachtin plus oil were 9.5 and 0.02 ppm respectively), but LC50 of Kingbo alone and Kingbo with oil were 8.86 and 3.13 ppm respictively. Laboratory bioassay showed that toxicity of azadirachtin was significantly increased when it was applied in an oil mixture. Thus, the addition of mineral oil (25%) to azadirachtin and Kingbo concentrations caused to increase the effectiveness of the insecticides and also could reduce up to 50% of insecticide applied dosages.

Keywords:*Azadirachtin, Matrine, LC50, Liriomyza sativae, Plant oil*

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Sublethal effect of two conventional insecticides on life table parameter of ectoparasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae)

Ahmad Baghdadi[1], Hassan Morsali[2]

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This study was performed to assess the effect of some sub lethal concentrations of Indoxacarb and Thiodicarb on life table parameters of H.hebetor (Say). The experiments was assessed at 27± 0.2°c, 65%±10% RH and a photoperiod of 16: 8 (L: D) hours. Our results indicated that in control, the first mortality was occurred after 26 days of appearance, and in other groups ,indoxacarb and thiodicarb, this phenomenon was occur at the days of 8 and 15, respectively. lx in control, indoxacarb and thiodicarb were 49.52, 43.77, and 40.55, respectively. The values of life expectancy (ex) showed the highest between all tested concentrations. In control was 39.46 while this parameter in indoxacarb and thiodicarb were 34.36 and 30.03.The Gross reproductive rate was significantly higher in control, to compare with insecticides treatment. The Intrinsic rate of increase in control was higher than other insecticide treatments. This parameter in control was 0.25573 while in insecticide treatment, Indoxacarb and thiodicarb, were 0.23864 and 0.24707 respectively. Other life table parameter such as finite rate of increase (λ) and net reproductive rate (R0) in control was higher. Mean generation time (Tc) in thiodicarb treatment was lowest and in indoxacarb treatment was the highest. Doubling time (Dt) didn't showed significant differences at all tested concentartions.

Keywords:*Life table parameters, sublethal concentration, Habrobracon hebetor, insecticides*

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Fauna of the family Laelapidae (Acari: Mesostigmata) in apple orchards of Salmas region, west Azarbaijan Province, Iran

Mohammad Hossein Kazemi[1], Sedigheh Alizadeh[2], Davood Shirdel[3], Heidar Adldoost[4], Nouraddin Shayesteh[5]

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The Laelapidae is a large family of worldwide distribution. Many of its species are ectoparasite of mammals, arthropods and birds. Some of laelapid mites are free living predators in soil habitats. During 2010-2011, they were collected and identified in soil and plant debris of apple orchards of Salmas region in West Azarbaijan Province. The mites were extracted with a Berlese/Tullgren funnel. They were cleared in Nesbitt's solution and were mounted in Hoyer's medium on microscope slides. In this study, 14 species were identified. Among them, four species were new for West Azarbaijan Province fauna and one species was new record for Iran. They were marked with one and two asterisks as follows: 1- Androlaelaps casalis (Berlese, 1887) 2- Euandrolaelaps karawaiewi (Berlese, 1903) 3- Eulaelaps stabularis (Koch, 1839) 4- Cosmolaelaps near gladii (Karg, 1993) 5- Cosmolaelaps lutegiensis* (Shcherbak, 1971) 6- Cosmolaelaps vacua (Michael, 1891) 7- Gaeolaelaps aculeifer (Canestrini, 1883) 8- Gaeolaelaps nolli (Karg, 1962) 9- Gaeolaelaps queenslandicus (Womersley, 1956) 10- Laelaspis missouriensis* Ewing, 1909 11- Ololaelaps sellnicki** Bregetova & Koroleva, 1964 12- Pneumolaelaps asperatus (Berlese, 1904) 13- Pneumolaelaps linteyini* (Smasinak, 1964) 14- Pneumolaelaps lubrica* Voigts & Oudemans 1904

Keywords:*Acari, Apple trees, Laelapid mites, Iran*

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Some of the mesostigmatic mites (Acari: Mesostigmata) in apple orchards of Salmas region, West Azarbaijan Province, Iran

Mohammad Hossein Kazemi[1], Sedigheh Alizadeh[2], Davood Shirdel[3], Heidar Adldoost[4], Nouraddin Shayesteh[5]

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Mesostigmata is a large assemblage of cosmopolitan mites that are usually free-living predators. During 2010-2011, the mites of Mesostigmata were collected and identified in soil and plant debris of apple orchards of Salmas region in West Azarbaijan. The mites were extracted with a Berlese/Tullgren funnel. They were cleared in Nesbitt's solution and were mounted in Hoyer's medium on microscope slides. In this study, 23 species belong to 19 genera and 13 families were identified. Among them, two families, three genera and 9 species were new for West Azarbaijan Province fauna. They were marked by one asterisk (*) as follows: Ameroseiidae: Ameroseius sp.; Kleemania plumosa Domrow, 1974; Ascidae: Antennoseius sp.; Arctoseius cetratus (Sellnick, 1940); Cheiroseius* nepalensis* Evans & Hyatt, 1960; Protogamasellus near massula (Athias, 1986); Blattisocidae: Lasioseius yousefi Athias-Henriot, 1957; Digamasellidae: Dendrolaelaps zwoelferi Hirscham, 1960; Eviphididae: Alliphis siculus (Oudemans, 1905); Macrochelidae: Macrocheles merdarius* (Berlese, 1889); Pachylaelapidae: Olopachys* sp.; Pachylaelaps karawaiewi* Berlese, 1929; Pachylaelaps resinae* Karg, 1971; Parasitidae: Parasitus hyalinus* (Willman, 1949); Parasitus near loricatus (Wankel, 1861); Parasiyus mycophilus* (Karg, 1974); Rhodacaridae: Rhodacarellus epigynialis Sheal, 1956; Veigaiidae*: Veigaia* planicola* Berlese, 1882; Uropodidae: Uropoda orbicularis (Muller, 1776); Trematuridae: Trichouropoda elegans Kramer, 1882; Nenteria stylifera Berlese, 1904; Dinychidae*: Uroobovella fimicola* (Berlese, 1903); Uroobovella marginata* (Koch, 1839).

Keywords:*Acari, Apple trees, Mesostigmatic mites, Iran*

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Effects of silver nanoparticles (Ag NPs) and polymers on stored pests for improving the industry of packaging foodstuffs

Somaye Allahvaisi

Islamic Azad University, Sanandaj Branch, Young researches Club, Kurdistan, Iran

Changes in agricultural technology have been a major factor shaping modern agriculture. Nano-silver is used in an increasing number of products. The customers`s complaints of the contaminations of foodstuffs to stored pests such as insects, mold and toxic fungi polluted packagings after a short time of storing, were reasons to perform this research. Cereals are one of the most important stored products. Microflora of wheat, corn, barley seeds mostly consists of bacteria, actinomycetes and fungi genera specially Aspergillus spp as amounts of aflatoxin B1 were detected in much cereals packaged. In this work, we conducted batch experiments to assess the efficiency of silver nanoparticles incorporated into packaging polymer on the growth of fungi and insect pests. Sitotroga cerealella Ol. (Lep.: Gelechiidae) is tested because of economical importance of this pest in storing`s industry of packaged cereals. Then, we applied Ag Nps on the best packaging polymer (PP). The results showed that not only Ag Nps could optimally omit toxic pathogens but also have a toxicant effect for insects. Thus, in this study the property of anti microbial and toxicity of Ag Nps as a non-chemical pesticide investigate in the terms of storing cereals foods. This study helps the health of foodstuffs after packaging until consuming.

Keywords:*Polymer, Aspergilus flavus, Penetration, Ag Nps, Packaging, pathogen*

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The investigation of different polymers resistance against penetration of *Rhyzopertha dominica* F. (Col. Bostrychidae) and CO2 gas

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In response to on-going consumer complaints regarding insect infestation of cereals-based packaged products kept at warehouses, research was undertaken. In order to the significance of cereals in feeding humans and the great application of polymers in packaging of these crops, we investigated the current polymers in market. Contaminated grains with lesser grain beetle adult released in around of the prepared packages with three kinds of transparent and flexible polymers followed: PE, PP and Cellophane in two thickness of 16.5 and 29 µm and so, determinate the permeability rate of polymers to this pest. The tests repeated 5 times and the means compared with MSTATC software and T-student test with 0.05-error level. Results showed that ten days after initiation of experiment the insects could create many punctures in the PE packages with thickness of 16.5 µm and the crop contamination was 100%. Whereas, pollution did not occur in no thicknesses of PP packages, at all. Comparing the means showed that difference of pest penetration percentage and crop contamination inside other polymers was significant in probability level 5%. So, PE with 16.5 µm thickness was worst polymer for packaging. In the other hand, because of great using fumigants for killing pests inside packages in stores, the used polymer for packaging in addition of protection of the crops, should be permeable to gases. Therefore, these polymers permeability to Co2 gas studied. The results showed that the polymers permeability to Co2 gas follow: Cellophane>PE>pp and the resistance of them against pest attack follow: PP>Cellophane>PE.

Keywords:*penetration, polymer, gas, Rhyzopertha dominica*

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Study of different polymer coverings for reducing the contaminations of cereals packagings in stores

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With regard to ever-increasing growth of crowd, keeping and protecting of stored products and raising of expiration longevity of them is the most important problem for our in future. In food industry, packaging is one of important factors in protection of quality and safety food. Prepared packaging from three transparent and flexible polymers PE, PP and Cellophane with 2 thicknesses of 16.5 and 29 µ, used for foodstuffs packaging. Insects of Tribolium castaneum Herbst. (Col: Tenebrionidae) and Sitophilus granarius L. (Col: Curculionidae) are contaminated serious pests in storages of cereals. With regard to development stages of infested in storages adult or larvae of these insects as target pest, located in manufactured package by mentioned polymers with flour and kernels and determinated permeability balance of polymers with different thicknesses to this pest. Each test had five replications. Means of penetration rates were compared by T-test at 5% level. Results showed that twenty days after initiation of experiment average of permeability rates to PE polymer with 16.5 µm thickness had the most permeability against the tested pests, as the product was completely infested and a lot of punctures were created in them by insects. So, PE was known moat unsuitable packaging polymer. PP was the best packaging polymer because no penetration was observed in the thicknesses of so, this polymer. Comparison of the means indicated that there was a significant difference between contamination rates of products inside the used polymers and their thicknesses ($p \leq 5\%$). To sum, the results showed that

Keywords:*polymer, thickness, foodstuffs, stored pest insects*

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Application of microwave radiation for controlling Tobacco beetle and studying packaging polymers to preserve cereals

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Despite application of protectant insecticides and fumigants, we observe the contamination of agriculture products in stores. Therefore, it is necessary to use a novel and suitable alternative control method. Developmental stages of Lasioderma serricorne F. including egg, larvae, pupa and adult were exposed to microwave radiation (2450MHZ) at power level of 150W with exposure times of 720, 420, 240, 120 Sec and with power level of 300W, and times of 100, 70, 40, 25Sec were for each developmental growth stage, separately. Experiments were carried out in complete randomized design with five replications and comparing mortality means was conducted in Duncan test with 0.05-error level. The mortality was determined at known intervals every day. The results showed that there was a relationship between constant power and exposure time. Complete mortality for eggs gained at 300W and 100Sec. Last instar larvae were controlled by 300W at 40Sec and for pupae, 150W at 420Sec were sufficient for not produce adults. In the case of adults, complete mortality was obtained by 300W at 70Sec. In these experiments, pupa was the most vulnerable and egg was the least susceptible. Therefore, 300W and 100Sec was sufficient for controlling all developmental stages of this insect. Radiation for disinfestations is effective when suitable packaging materials pack the product. PP was the best packaging material because the insect wasnot able to puncture it and created infestation. The most unsuitable packaging was Cellophane because

Keywords:*pest control, microwave radiation, foodstaffs, polymer*

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Estimating of phosphine (PH3) gas effective quantities on different development stages *Plodiainterpunctella* (Hb.) in passing from nut packages in store

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Sub lethal doses cause to resistant the pests in short times. Therefore, determining values of used pesticides in stores is serious that these are able to pass packaged materials and to fumigant the products inside packaging. This pest in addition of high damage in stores, caused the favorable conditions for attack and growth of pathogenic agents as example fungi that many of them infected the foodstuffs with secretion of toxic materials such as aflatoxine, severely and create dangerous diseases on human and livestock such as cancers. Therefore, challenging to this pest is important from agriculture and sanitation aspect. In this research, we place 15 gr of infected nuts with three different development stages of Indian moth (egg, 9 days old larvae and adult) inside polymeral packages of PP (suitable for packaging in stores). Fumigation was conducted by use of aluminum phosphide tablets 56% in vacant air steel tanks. The tests repeated five times in 5 treatments with control test. After 72 hours, the mortality accounted. The obtained data was analyzed using probit procedure of SPSS10 software. Under these conditions, the respective LC50 and confidence limits values to kill 50% of inoculated eggs, larvae and adult were 36.54(27.5?48.8), 8.75 (6.5-11.6) and 3.9 (2.9?5.14) mg Al/L, respectively. The most mortality was for 417.85 mgL-1 concentration. In conclusion, the results demonstrated that eggs were more resistant than larvae and adults in this species and those adults were the most susceptible stage. Also, the best stage of combating with Indian moth is adult stage.

Keywords:*packaging, PH3, Plodiainterpunctella, penetration,fumigation*

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The investigation of protein effects on fertilization *Tribolium castaneum* (Col.: Tenebrionidae) in wheat stores

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Proteins are one of the most important sources created energy in insects' body cells. Studying this matter how much of protein affect on pest population is serious. In this research, we separate the female and male adult insects of Tribolium castaneum Herbst. and added some creatine to indispensable wheat flour for insect feeding at 19:1, 14:1, 9:1 and 6:1 ratios. The tests repeated five times and did for seven continuous generations in warehouse at 25±5°C with 70% Rh and with control test (without decrease or increase of protein). Means compared by in MSTATC software, Dancan test with 0.05-error level. The results showed that at 6:1 ratio from the wheat flour-creatine compound, decreased fertilization and population growth significantly ($p \leq 5\%$) in 7th generation to ½ of 1th generation so much that sometimes did not lay any egg. Finally, this research carried out on the wheat grains had 14% loss of sunn pest and their protein was lost. In order to the results, needed protein for red flour beetle cause proper activity but too increasing of its caused toxicity and deduction of progeny. So, fertility rate and population growth rate (rm) of one species of stored-pest depended on the compounds percent of formed foodstuffs. Thus on basis of protein value, rm increase of T. castaneum followed: well wheat>wheat with decreased protein>wheat with increased protein.

Keywords:*fertilization, Tribolium castaneum, toxicity, storage conditions*

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Oral toxicity of entomopathogenic symbiotic bacterium *Photorhabdus temperata* against various insects and nematodes

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Photorhabdus temperata is a symbiotic bacterium that mutually associated with entomopathogenic nematode Heterorhabditis. The oral toxicity of 5 P. temperata strains collected in different regions of Korea was determined in the in the immature and mature stages of various insects and nematodes. When diet or water containing culture media containing 1 of the 5 different strains was ingested by immature insects, the first instar larvae of both Galleria mellonella and Lucilia caesar and young larvae of Culex pipiens pallens died within 3-5 days after treatment. Particularly, a fraction separated by ethyl acetate was highly toxic to C. pipiens pallens nymphs. However, mortality of Plodia interpunctella neonate larvae was slightly slower and reached 94.4%-100% within 7 days after treatment. The mortality rate of a control group given a diet containing water, the medium without cultured bacteria, or Escherichia coli culture medium was not affected. The oral ingestion of sucrose diet solutions (20%) containing supernatant of the Photorhabdus culture media was completely lethal at 60 h after treatments. In addition, ingestion of Photorhabdus culture media significantly inhibited molting of third instar Plodia larvae and fecundity of a katydid, Paratlanticus ussuriensis. Soil treatment of Photorhabdus culture medium was lethal to a rootknot nematode, Meloidogyne arenaria, and inhibited rootknot formation in cucumber and tomato plants. Our results suggest that the oral administration of Photorhabdus bacterial medium was highly lethal to immature insects and also toxic to the development and reproduction of various insects.

Keywords:*Biopesticides, Developmental inhibition, Root-knot nematode, Oral toxicity, Photorhabdus temperata.*

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Evaluation of *Solenostemma argel* extracts for their potential larvicidal activities against *Anopheles arabiensis* Patton (Diptera: Culicidae)

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Among the prevalent mosquito species in Africa Anopheles arabiensis is the important malaria vector distributed in most countries. The main control tactics adopted depend largely on the application of synthetic chemicals. However, the socio-economic and environmental drawbacks of these chemicals are well known in everywhere. Therefore, this work was proposed to study the occurrence of A. arabiensis in central semi arid region of Sudan, and to evaluate different extracts of Solenostemma argel on its control. Leaves and seeds of the plant were extracted using water and organic solvents (viz., petroleum ether and ethanol), and their phytochemical constituents were analyzed. Bioassays of such extracts were conducted against the 4th instar larvae of the mosquito, where the mortality and residual effects were compared. The results revealed that the relative occurrence of A. arabiensis were higher around the River Nile. The chemical constituents of the tested extracts showed variable levels of secondary metabolites, i.e., alkaloids, saponins, flavones, flavonoids, amino acids, triterpenoids and sterols. The highest mortality percent (90%) was achieved by the seeds petroleum ether extract (LD50 1000ppm), which was comparable with that of the standard insecticides (Malathion and Abate) used. This extract also gave the best residual activity after one month. However, the mortality means of different botanical treatments increased with increasing concentrations and time of exposure. In conclusion, the findings proved the potent insecticidal activity of S. argel against A. arabiensis, and suggested more studies to be utilized commercially as natural mosquito larvicide.

Keywords:*Solenostemma argel, Anopheles arabiensis, larvicide, mortality, residual effect.*

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Effects of thiamethoxam on feeding and tunneling activities of the termite Microcerotermes diversus Silvestri (Isoptera: Termitidae) under laboratory conditions

Zeinab Sarah Fathollahi
Shahid Chamran University of Ahwaz, Iran

Treating soil with insecticides is thought to be an effective method for termite control. Speed of action and repellency of insecticides are important factors for discussing their barrier effects as soil-treatment termiticide. Microcerotermes diversus (Silvestri) is the most economically destructive termite in structures in Ahwaz region, because the species feed on anything with cellulose. M. diversus live in subterranean nests and access to buildings and plants through subterranean galleries. The chemical barrier effect of thiamethoxam against M. diversus was evaluated under the laboratory using glass tube and plastic container methods. The concentrations used ranged from 5 to 5000 ppm. Soil treated with >50 ppm of thiamthoxam had a barrier effect and could stop the penetration of termite within 7days. Thiamethoxam did not act as a repellent, as indicated by a gradual increase in mortality at >50 ppm with slight penetration, tunneling area and feeding activity into the treated soil during the 7-day trial. The results suggest that thiamethoxam is an effective termiticide, a suitable barrier for soil treatment, that also acts as a non-repellent, inhibitive entry of termites into treated soil.

Keywords:*Microcerotermes diversus, Thiamethoxam, Repellent, Mortality, Tunneling and Feeding Activities*

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Transfer of thiamethoxam from exposed to non-exposed workers of *Microcerotermes diversus* Silvestri (Isoptera: Termitidae)

Zeinab Sarah Fathollahi
Shahid Chamran University of Ahwaz, Iran

Because Microcerotermes diversus (Silvestri), a subterranean termite, is able to attack cellulosic materials, is destructive to structures, and is the most economically important wood pest in Iran. Therefore, control or management of the termite is of serious public concern. The use of nonrepellent termiticides such as thiamethoxam (a neonicotinoid compound) has been gaining popularity in recent years. The success of these compounds in managing termite infestations depends primarily on their speed of action and non-repellent characteristics. The current study was designed to determine termite mortality, non-repellency, and horizontal transfer of termiticide by trophallaxis when termites were in contact with treated-filter paper in laboratory choice and no-choice tests. A donor-recipient trial in two ratios was used: 1:1(10 donors: 10 recipients) and 1:5 (10 donors: 50 recipients). In one trial, workers were placed on filter paper previously treated at 5.0, 10.0 or 15.0 ppm thiamethoxam. Exposed workers were then removed from the treated paper, placed in a clean container, and then allowed to have side-by-side feeding interactions with previously non-exposed recipient workers for 14 days, after which mortality was assessed. Results indicated that mortality of non-exposed workers contacted by the exposed workers increased with increasing insecticide concentrations in both donor-recipient worker ratios. Therefore, transfer of lethal insecticide concentrations from the donor to recipients was observed with thiamethoxam.

Keywords:*Microcerotermes diversus, Thiamethoxam, Repellent, Mortality, Tunneling and Feeding Activities*

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Differential response of male and female adult banana weevil, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) to fresh and rotten host cues

Kamala Jayanthi Pagadala Damodaram[1], Vivek Kempraj[2], Ravindra Aurade[3], Abraham Verghese[4]

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The banana pseudostem weevil, Odioporus longicollis Olivier (Coleoptera: Curculionidae) is an economically important pest in South East Asia limiting the production of banana. The pseudostem becomes weak and starts rotting, leading to break down upon infestation. Management practices to control the damage caused by weevil vary widely from regular application of chemical insecticides, cultural practices and biological control. Attempts to control this monophagous weevil through methods involving semiochemicals merits more attention as they provide selective control. In our search for an effective phyto-semiochemical from host plant, banana, an interesting sex linked differential response of weevils to banana volatiles was noticed. Y-tube bioassy was carried out to confirm this preference. When the host volatiles were compared with control, male and female weevils exhibited preference for both fresh and rotten stem volatiles. However, in choice bioassay, the female adult weevils showed a clear preference for rotten stem volatiles and males to fresh stem volatiles showing a clear sex linked preference for host cues. Further studies to identify the responsive chemical fraction that elicited response were identified through GC-EAD and GC-MS.

Keywords:*GC-MS, GC-EAD, Odoiporus longicollis, volatiles, banana*

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Trichome based defense in different cultivars of brinjal on neonates of Brinjal fruit and shoot borer, Leucinodes orbonalis

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The Brinjal fruit and shoot borer, Leucinodes orbonalis Guenee (Pyralidae: Lepidoptera) is one of the major insect pest on cultivated brinjal (Solanum melongena L.) causing heavy fruit loss. In the present study, attempts were made to understand the role of trichome based host-plant resistance to L. orbonalis mainly in different wild cultivars of Solanum viz., S. macrocarpon, S. indicum, S. viarum, S. torvum, S. ethiopicum, S. mammosum, S. gilo compared to cultivated S. melongena. A total of eight types of glandular and non-glandular trichomes were observed in wild as well as cultivated Solanum species. There is difference in the total number as well as types of trichomes present in adaxial and abaxial surface of brinjal leaves. The stellar trichomes were found to be abundant in S. melongena, S. indicum and S. gilo. Whereas, in S. mammosum and S. viarum Type I - Type VII trichomes were also present besides stellar trichomes. Laboratory bioassays were also carried out to study the influence of these trichomes on neonate larvae of L. orbonalis. In, S. mammosum and S. viarum, maximum larval mortality was observed within 4?6 h after larval release compared to other cultivars where it was found to be up to 10?12 h. The entrapment of larvae was also found to be very high in S. mammosum and S. viarum compared to other cultivars. However, the initial repellency was observed to be high in S. indicum, S. torvum and S. gilo. The glandular trichome exudates may be playing important role in causing

Keywords:*trichome, solanum, Leucinodes, brinjal, phenols, flavonoids*

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Whitefly resistance in tomato

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Whitefly resistance is a much desired trait in tomato growing. An F2 population was studied for resistance against whitefly Bemisiatabaci by analyzing phenotypic and metabolic traits. Multivariate statistical analyses revealed that a large complex of metabolic components was associated with whitefly resistance and a smaller number of metabolites were associated with whitefly susceptibility. Minor QTLs were identified for whitefly phenotypic parameters and metabolic constituents that associated with whitefly resistance and susceptibility. In an F2BC1 population we observed that some genotypes were still completely resistant against whiteflies, but the complexity of metabolic profiles was strongly reduced and only a selection of resistance-related constituents was present. A QTL analysis of metabolic constituents associated with whitefly resistance revealed the presence of metabolic QTLs that co-localize with whitefly phenotypic QTLs. Plant morphologic characteristics were studied and a positive correlation was found between the presence of type I and IV glandular trichomes on the leaf surface and reduced vitality and oviposition of the whitefly. In addition, it was observed that the presence of type I and IV trichomes was positively correlated.

***Keywords**:resistance*

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Toxicological potential of peel extract of *Citrus limon* against *Tribolium castaneum, Sitophilus granarius* and *Cryptolestes ferrugineus* under laboratory conditions

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Laboratory experiments were conducted so as to evaluate the effectiveness of Citrus limon peel extracts on Tribolium castaneum, Sitophilus granarius and Cryptolestes furragenius. Experiments were successfully completed in the Stored Grain Pest Management Cell, Department of Agri. Entomology, University of Agriculture, Faisalabad. The toxicological potential of peel extracts was evaluated at different exposure periods. Three concentrations (5, 10 and 15 %) of the C. limon peel extract were used and results were evaluated after different exposure periods (24, 48, 72, 96, 120, 144 and 168 hours). Residual method was used in the experiment to determine the contact toxicity of peel extract. The results showed that C. furragenius showed the best response followed by S. granarius and T. castaneum, respectively. Overall C. furragenius showed 93.01%, 69.78% and 61.96% mortality, S. granarius showed 55.57%, 35.835% and 28.21% and T. castaneum showed 23.53, 16.77, 16.47% mortality at 15, 10 and 5% of concentrations, respectively. C. furragenius showed the maximum mortality of 100% at 15% concentration in 120h exposure. S. granarius showed 95.68% mortality at 15% concentration in 168h exposure whereas T. castaneum showed a maximum of 64.02% mortality at 15% concentration in 168h exposure. The LC50 values were also calculated according to Probit analysis method. After an exposure of 7 days, the results were evident that acetone peel extracts of C. limon has a strong potential to control primary as well as the secondary pests of stored grains and can serve as an eco-friendly bio-pesticide.

Keywords:*Lemon Peel Extract, bio-pesticide, Mortality, Stored grain insects*

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Phytochemical analyses of five sudanese plants for their constituents of biologically active compounds

Abdalla Abdelrahim Satti[1], Amal Elsayed Edriss[2]

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Higher plants are known to contain several types of secondary metabolites with profound biological activities. Such plant derivatives represent untapped sources for unlimited applications in public life and industry. Due to their diversified structural compounds, they also provide new models to produce chemical analogues with enhanced potencies and/or utilities. In agriculture, numerous plant extracts were proved significant effects in controlling different pests and diseases; hence some commercial pesticides were formulated from certain botanical compounds. In this research, phytochemical analyses of five indigenous plants were investigated. These plant species and their studied parts included; Citrus paradisi (leaves & seeds), Calotropis procera (leaves & flowers), Eucalyptus camaldulensis (leaves & fruits), Ocimum basilicum (leaves & seeds) and Ricinus communis (leaves & seeds). Three extracts (aqueous, ethanol and petroleum ether) were analyzed from each plant to ascertain their active chemical groups of non polar and polar components. The results of water extract showed that alkaloids, saponins, flavonoids and flavones are the main chemical groups present in all plants at variable levels. Moreover, traces amounts of sterols and triterpenes were detected in seeds of R. communis. However, ethanol extract was dominated by triterpenoids, alkaloids and sterols, and to some extent flavonoids and flavones. On the other hand, petroleum ether extract yielded principally triterpenoids and sterols. Since these plants are available in the country and having many traditional uses, particularly in medicine and pest control, the current research explained that promising active ingredients could be obtained from them for proper commercial and industrial exploitation.

Keywords:*Indigenous plants, secondary metabolites, phytochemical analysis, active compounds.*

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P7

Courtship and mating behaviors of *Phyllotreta striolata* (Fabricius) (Coleoptera, Chrysomelidae)

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Phyllotreta striolata (Fabricius) is one of the destructive pests to cruciferous crops worldwide. By employing real-time shooting techniques, the characteristics of courtship and mating behaviors between male and female adults were analyzed. Specifically, we found that the courtship behavior cost no more than 10 seconds and its frequency showed an increasing trend after 15:00:00 PM. However, this mating behavior, which lasted generally from 13 to 15 minutes, had a low frequency that was only once or twice per day. In addition, there was no correlation between the accomplishment of mating behavior and the multi-frequency of preludiously successional courtship behavior.

Keywords:*Phyllotreta striolata (Fabricius), courtship behavior, mating behavior*

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Fumigasion and packaging integrated effects for reducing *Callosobruchus maculates* F. (Col: Bruchidae) contaminathions in stores

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Protection of stored gricultural products such as grains in bages because of its warps make it possible to penetrate the pest insects. Prepared packages from three transparent and flexible polymers including; PE, PP and Cellophane with 2 thicknesses of 16.5 and 29 µ.m., used for grains packaging. Adult insects of Callosobruchus maculatus F. (Col: Bruchidae), as target pest, located around of manufactured package by mentioned polymers with grains and determinated polymers permeability to this pest. Each test had five replications. Means of penetration rates were compared by MSTATC and T-test at 5% level. Results showed that nine days after initiation of experiment average of permeability rate to cellophane polymer (16.5 µ.m. thickness) was %10±0.2. While no penetration were observed in PP polymers. Comparison of the means indicated that there was a significant difference between permeability and contamination rates of products at different polymrs and thicknesses ($p \leq 5\%$). Therefore, cellophane was less suitable polymer for packaging of foodstuffs. On the one hand, when suitable packaging for control of pest is efficacious that products placed inside package is none contamination. Thus, permeability to Phosphine gas (PH3) in packaging was considerated.

Keywords:*fumigation, polymer, penetration, pest insects, thickness*

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Others

P7

Efficacy assessment of *Isaria fumosorosea* and SilicoSec against *Tribolium castaneum*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus* and *Liposcelis paeta*

Waqas Wakil[1], Muhammad Yasin[2], Yong Jung Kwon[3], Tahira Riasat[4], M. Usman Ghazanfar[5], Mirza Abdul Qayyum[6]

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The present research was aimed to evaluate the insecticidal efficacy of entomopathogenic fungi, Isaria fumosorosea and diatomaceous earth formualtion SilicoSec against four species of stored grain insect pests; Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), Rhyzopertha dominica (F.) (Coleoptera: Bostrychidae), Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeidae) and Liposcelis paeta (Pearman) (Psocoptera: Liposcelididae). The fungus was admixed with wheat grains at 1.7×106 and 1.7×108 conidia kg-1 individually and with 200 ppm of SilicoSec. The tests were conducted at 30±1oC and 65±5% rh. The adult mortality of the exposed insects was measured after 7, 14 and 21 d of exposure. The mortality for all the insects treated with the highest dose of I. fumosorosea with SilicoSec was significantly higher than the alone treatments. The mortality was increased with exposure interval and increased dose rates. The rate of mycosis and sporulation in the cadavers of all the insects was higher where the I. fumosorosea was applied at low dose rates.

Keywords:*Isaria fumosorosea, diatomaceous earth, wheat, stored grain insects*

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Others

P7

Evaluation of biocontrol agents for the control of fruitborer *Helicoverpa armigera* (Hubner) in the fields of tomato crop

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The study was conducted to assess the efficacy of various biorational microbial pesticides, Beauveria bassiana (1.3x108 conidia/ml), HaNPV (2.5x107 POB/ml), Deodonia viscose (7 ppm), new chemistry Coragen (99 ml/ha) and their possible combinations against Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) on tomato crop under various ecological conditions. The treatments were sprayed twice, the data on larval population, percent fruit infestation, population of natural allies and yield of tomato fruits were recorded. The treatments showed significant difference, however, the application of HaNPV+Coragen showed maximum control of pest followed by B. bassiana+Coragen and the insecticide treatment alone. However, the minimum pest control was recorded in those plots where plant extract was sprayed, the percent fruit infestation in all the six pickings differs significantly among treatments. The application of HaNPV+Coragen showed minimum pest infestation and was at par with those plots where B. bassiana+Coragen were sprayed. The application of plant extract showed maximum fruit infestation and did not proved an effective treatment. The results clearly indicated that the microbial insecticides may provide better control of the insect pests without harming the natural allies and the ecosystem.

Keywords:*B. bassiana, HaNPV, plant extract, new chemistry insecticide, fruitborer, tomato*

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Others

P7

Demographic parameters of cowpea aphid, *Aphis craccivora* (Homoptera: Aphididae) reared on botswana cowpea landraces

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The variation demographic parameters were used to assess resistance of cowpea landraces against of the cowpea aphid (Aphis craccivora Koch). The cowpea landraces were B 261-B, B 383, B 013-F, B 339 and susceptible variety blackeye was included as a check. The demographic parameters studied were survival and fecundity, intrinsic natural rate of increase, pre-reproductive period and relative growth rate of cowpea aphid. The demographic parameters of A. craccivora differed significantly among varieties supporting our prediction that cowpea landraces resistant to cowpea aphid would have significant effect demographic parameters of the aphid. We observed significantly lower survival, fecundity, intrinsic natural rate of increase and relative growth rate on landraces B261-B and B 383 compared to other landraces. A significant delay in pre-reproductive period was also observed on B261-B and B 383. The results showed that landrace B261-B and B 383 have increased level of resistance as shown by delayed pre-reproductive and reduction in population growth rate, survival and fecundity. This information will be used in the characterization of the landraces before been incorporated in the breeding programme in Botswana.

Keywords:*Landrace, survival, resistance, demographic, life table*

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Others

P7

Effects of levels of initial infestation on damange to registant and susceptible cowpea by *Callosobruchus maculatus* (Fab.)

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[1]Rufus Giwa Polytechnic, Nigeria, [2]Federal University of Technology, Nigeria, [3]Federal University of Technology, Nigeria

Cowpea cultivars Ife Brown and IT84S-2246-4 were screened for resistance to Callosobruchus maculatus (F.) attack using different insect densities. The number of eggs laid on the seeds, number of seed bearing eggs and seed without eggs, mean number of eggs per seed, number of F1 progeny, number of seed with holes and seed without holes, mean number of seed with hole and developmental time increased as insect population increases. Significantly, more eggs were laid on the seed of Ife Brown (Susceptible cowpea variety) than IT84S-2246-4. However, low adult emergence and high development period were observed on IT84S-2246-4 indicating clear cultivar difference at all insect population. The assessment of the first filial generation (F1 progeny) of the two varieties showed further, that oviposition reduced in insect that emerged from IT84S-2246-4 (resistant variety) when reared on Ife Brown cowpea seeds. Irrespective of cowpea varieties used in the experiment, egg laid and adult emergence by C. maculatus significantly increased with increase in insect density.

Keywords:*cowpea varieties, filial generation, oviposition, insect densities, seed bearing eggs*

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Others

P7

Diversity of non-target insect community in bt and non-bt cotton

Anjum Suhail[1], Muhammad Arshad[2], Zain ul Abdin[3], Muhammad Dildar Gogi[4], Rashid Rasool Khan[5]

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Cotton crop hosts a rich diversity of insect predators and parasitoids, which have the significant role in regulating the pest population. Bt cotton can alters the insect diversity especially predators and parasitoids by reducing the abundance of target lepidopterous species. A field study was planned for two cotton seasons to compare the non-target insect diversity among transgenic Bt-cotton and non Bt cotton under sprayed and unsprayed conditions. The experiments were conducted at Postgraduate Agriculture Research Station (PARS), Faisalabad, Punjab, Pakistan. A variety of sampling methods were used including sweep net, drop cloth and pitfall trap. The collected insect fauna were identified and further grouped into four categories: non-target pests, beneficials (predators and parasitoids), pollinators and visitors (non cotton insects). The results indicated significant differences in the abundance and species richness of insect community and sub-communities (pests, beneficials, pollinators and visitors) among the treatments across each sampling techniques (sweep net, drop cloth and pitfall) during both years. The insecticide applications significantly reduced the abundance and species richness in sprayed plots of Bt and non-Bt cotton but no significant differences were found between unsprayed Bt and non-Bt cotton, which showed that Bt cotton has no adverse affects on the abundance and richness of non-target insect community and sub-communities. The diversity indices (species diversity and evenness) were generally higher in sprayed plots of Bt and non-Bt cotton. The differences in diversity indices among the varieties in sprayed and unsprayed conditions showed that insecticide application had adverse effects on rare species.

Keywords:*Biodiversity, Bt cotton, insect beneficial, Pakistan*

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Others

P7

Studies on aphid pests of apple in orchards of Himachal Pradesh state of India

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Apple is an important fruit crop of Himachal Pradesh state of India. Green apple aphid, Aphis pomi De Geer and woolly apple aphid, Eriosoma lanigerum (Hausmann) are considered as the serious pests damaging the apple plants severely in all the apple growing regions of this state. Survey of the apple nurseries as well as apple orchards of this region revealed that the populations of Aphis pomi occurring on apple nursery plants are kept under control by a number of predators belonging to Coccinellidae and Syrphidae. The larvae and adult coccinnellid predators observed feeding on Aphis pomi populations in Himachal Pradesh are Cheilomenes sexmaculata (F.), Coelophora bissellata Mulsant, Coelophora saucia (Mulsant) and Harmonia dimidiata (F.) and the syrphid larvae recorded feeding on green apple aphid are Betasyrphus serarius (Weidemann), Episyrphus balteatus De Geer and Metasyrphus confrater (Weidmann). The biology and feeding potential of these prerdators of Aphis pomi, have been investigated in detail and are presented in this paper. The observations on the biological control of woolly apple aphid are still in progress. The present paper includes the detailed information on the various aspects of life history of these two aphid pests of apple in Himachal Pradesh, India and also on the biology and feeding potential of predators of Aphis pomi on apple nursery plants in this region. The findings may prove useful in developing the effective strategies for the management of these aphid pests in this region.

Keywords:*Apple, Green apple aphid, woolly apple aphid*

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Others

P7

Phytosanitary risk of bruchids in import of legume germplasm in India

Shashi Bhalla[1], Manju Lata Kapur[2], Kavita Gupta[3], Charan Singh[4], Prakash Chandra Agarwal[5]

[1]National Bureau of Plant Genetic Resources, New Delhi, India, [2]National Bureau of Plant Genetic Resources, New Delhi, India, [3]National Bureau of Plant Genetic Resources, New Delhi, India, [4]National Bureau of Plant Genetic Resources, New Delhi, India, [5]National Bureau of Plant Genetic Resources, New Delhi, India

Import of legume germplasm for crop improvement is associated with quarantine risk of exotic bruchids which move across the countries as hidden infestation in seed. Import of planting material in India is regulated under Plant Quarantine (Regulation of Import into India) Order 2003 and National Bureau of Plant Genetic Resources is the nodal organization authorized to issue Import Permit for germplasm including transgenics meant for research and undertake their quarantine processing. The import of 140 legume genera germplasm carries the phytosanitary risk of over 500 bruchids into India. The quarantine risk is much higher due to their high economic significance, wide host range, adaptability and possibility of presence of biotypes in some bruchids. During last 35 years, ~70,000 imported samples of legumes were subjected to soft X rays generated generally at ~22Kv, 3 mA at a distance of 30 cm from the source for 30 seconds to detect and exclude hidden infestation. This resulted in detection of bruchid infestation in 5,823 samples representing 33 species of bruchids including 25 alien ones infesting > 35 legumes. Some of the bruchids intercepted from different countries but not yet reported from India included Bruchidius atrolineatus in Vigna unguiculata; Bruchus dentipes in Vicia faba; B. ervi in Lens spp., Lathyrus latifolius, Vicia spp.; Callosobruchus subinotatus in Voandzeia subterranea; Merobruchus paquetae in Albizia guachepele; Mimosests mimosae and M. nubigens in Acacia farnesiana. Phytosanitary risk assessment and mitigation measures are therefore extremely important to facilitate safe import of plants and planting material for agricultural biosecurity.

Keywords:*Phytosanitary risk, quarantine, legumes, bruchids*

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Others

P7

Effects of gamma radiation on mature larvae of *Pectinophora gossypiella* (Saunders) and their F1 progeny

Syed Shahzad Ali

Sindh Agriculture University Tandojam, Pakistan

The mature larvae of Pectonophora gossypiella (Saunders) obtained from the laboratory culture maintained on casein wheat germ diet were irradiated at 35, 45, 55 and 65 Gy doses at a dose rate of 30.8 Gy/min. in a Cs-137 gamma irradiator. The pupation was delayed as the radiation dose increased. Furthermore, larval survival to pupal and adult stages were also susceptible to gamma radiation doses were increased. Females were more susceptible to gamma radiation than males. The effect of gamma radiation on reproduction in P1 months following irridation of mature larvae was dose dependent. As the dose of mature larvae increased, average egg production, hatch percentage and adult longevity reduced. Egg production, was reduced more drastically in the crosses Untreated Male x Treated Female (UTM x TF) and Treated male x Treated Female (TM X TF) than the crosses Treated Male x Untreated Female (TM x UTF). Complete sterility was recorded when treated males were paired with treated females at 45 Gy and higher doses of gamma radiation. The results on the egg production, hatch percentage and adult longevity of F1 progeny of male parents following irridation of mature larvae showed that egg production was reduced significantly in crosses UTM x F1 Female at 35 Gy and complete sterility was recorded at higher doses. In crosses F1 Male x F1 Female, complete sterility was recorded at all the test doses of gamma radiation. The radiation doses higher than 35 Gy were more lethal either in F1 Male x F1 Female.

Keywords:*Pectinophora gossypiella (Saunders), irradiation, Cs-137 gamma irradiator*

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Others

P7

Effect of different temperature regimes on the development of *Bracon hebetor* (Say) (Hymenoptera: Braconidae) and its parasitism

Rashad Rasool Khan[1], Muhammad Ashfaq[2], Sohail Ahmed[3], Khuram Zia[4], Muhammad Arshad[5], Muhammad Dildar Gogi[6]

[1]University of Agriculture, Pakistan, [2]University of Agriculture, Pakistan, [3]University of Agriculture, Pakistan, [4]University of Agriculture, Pakistan, [5]University of Agriculture, Pakistan, [6]University of Agriculture, Pakistan

The parasitizing ability and biology of a larval parasitoid; Bracon hebetor (Say) (Hymenoptera: Braconidae) were studied in an experiment to determine the optimum conditions for its mass rearing. It was observed in this experiment, that the host larvae were completely parasitized, by the parasitoid, at 25 ± 2 °C and 30 ± 2 °C of temperatures. However, at higher temperatures the parasitism as well as the growth of the parasitoid, were negatively affected. So it is concluded that the best ecological conditions, for the mass rearing of the parasitoid as well as its performance are 30 ± 2 °C of temperature, 65 ± 10 % R.H and 16:8 L:D, periods.

Keywords:*Temperature regimes, Mass rearing, Development, Parasitism, Bracon hebetor*

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PS7TH479

Others

P7

Behavior of inscet-pests and pathogens on the cones and seeds of *Pinus gerardiana* (Chilgozapine)

Pawan Kumar
Himalayan Forest Research Institute (ICFRE), Shimla, India

Pawan Kumar, RC Sharma and VRR Singh Abstract: Pinus gerardiana Wall. 1832, commonly called as Chilgoza pine, mostly grows in valleys at 2000-3350 m, amsl elevation, in the dry temperate forest of the inner ranges of the Himalaya, where the summer monsoon is weak and precipitation, mostly in the form of snow, rarely exceeds 1000 mm per year. Insects constitute one of major biological destructive agents in tree seed productivity. The export of Chilgoza seeds contributes appreciably to the annual income of the most of the families living in the areas of its distribution. In the district of Kinnaur (Himachal Pradesh) alone, the approximate value of export of its annual produce is around 18 Crore of rupees in a year. But the cone and seeds are now being attacked by variety of insect-pests and pathogens causing degradation in the quality of seeds and even loss of seed crop. Cones and seeds of coniferous tree species are heavily attacked by coneworms, Dioryctria abietella and Cateremna (Euzophera) cedrella (Lepidoptera: Pyralidae) in western Himalaya. Dioryctria abietella is responsible for causing economic losses in cones and seeds of various conifer species. In Chilgoja pine, reported almost 100% attack at Kalpa, Himachal Pradesh. Insect damage varies greatly, depending on age, tree species, location, and insect control plan. On occasion, orchards may lose their entire seed crop. Without seed, there can be no seedlings, and without pest management expertise, seed crops are at risk to damage or destruction caused by insects and diseases. Such damage could

Keywords:*Borer, Chilgoza, Cone, Seed, Lepidoptera.*

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PS7TH480

Others

P7

Isolation and identification of Alfalfa mosaic virus from Egypt

Sahar Hassan El Helaly[1], Amin Abo El-Ghar[2], Awad Mohamed Ahmed[3], Amal Farghaly[4]

[1]Menofia University, Egypt, [2]Menofia University, Egypt, [3]Menofia University, Egypt, [4]Menofia University, Egypt

Egypt is the most important of Mediterranean countries produced potato crop. Alfalfa mosaic virus is one of the most important viruses that infect potato. The aim of this study is: virus isolation from potato, virus identification using transmission methods, biological methods, serological methods, light microscope, electron microscope and molecular methods also measure the susceptibility of some local and imported verities and the effect of virus infection to some vegetative characters, physiological and chemical analysis of potato plants (chlorophyll, carbohydrates, protein, starch and mineral elements N,P and K).after isolation and identification we found that: Host range: Reaction to different hosts differed according to the host plant and virus isolate. Mode of transmission: Isolated virus was readily transmitted mechanically Insect transmission differed according to the species of aphid AMV easily transmitted by grafting Serological studies: Infected plants gave positive reaction with AMV antiserum Viral infection caused anatomical changes of infected tobacco plants using light and electron microscopy. All of local and imported cultivars were susceptible to the virus. AMV infection affect some of vegetative characters, photosynthetic pigments and chemical constituents. Finally the molecular characterization of the viral genome of the Egyptian isolates.

Keywords:*AMV, PCR, potato, Egypt,*

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PS7TH481

Others

P7

Behavior of inscet-pests and pathogens on the cones and seeds of *Pinus gerardiana* (Chilgozapine)

Pawan Kumar
Himalayan Forest Research Institute (ICFRE), Shimla, India

Abstract: Pinus gerardiana Wall. 1832, commonly called as Chilgoza pine, mostly grows in valleys at 2000-3350 m, amsl elevation, in the dry temperate forest of the inner ranges of the Himalaya, where the summer monsoon is weak and precipitation, mostly in the form of snow, rarely exceeds 1000 mm per year. Insects constitute one of major biological destructive agents in tree seed productivity. The export of Chilgoza seeds contributes appreciably to the annual income of the most of the families living in the areas of its distribution. In the district of Kinnaur (Himachal Pradesh) alone, the approximate value of export of its annual produce is around 18 Crore of rupees in a year. But the cone and seeds are now being attacked by variety of insect-pests and pathogens causing degradation in the quality of seeds and even loss of seed crop. Cones and seeds of coniferous tree species are heavily attacked by coneworms, Dioryctria abietella and Cateremna (Euzophera) cedrella (Lepidoptera: Pyralidae) in western Himalaya. Dioryctria abietella is responsible for causing economic losses in cones and seeds of various conifer species. In Chilgoja pine, reported almost 100% attack at Kalpa, Himachal Pradesh. Insect damage varies greatly, depending on age, tree species, location, and insect control plan. On occasion, orchards may lose their entire seed crop. Without seed, there can be no seedlings, and without pest management expertise, seed crops are at risk to damage or destruction caused by insects and diseases. Such damage could result in repercussions for future production and

Keywords:*Borer, Chilgoza, Cone, Seed, Lepidoptera.*

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